PLANT PHYSIOLOGY

VOLUME 15

1940

THE SCIENCE PRESS PRINTING COMPANY
LANCASTER, PENNSYLVANIA

TABLE OF CONTENTS

Number 1. January

Number 2. April

NUMBER 3. JULY

NUMBER 4. OCTOBER

ERRATA

VOLUME 15

Page 23, author's name, for "G. PRESTON" read C. PRESTON.
Page 26, table II, delete "gm." in column heading above 5.57.
Page 27, table III, heading, last column, for "per disc" read per 10 discs.
Page 53, line 5 from bottom, for "Some (19)" read Some (19) see.
Page 341, citation no. 6, for 6-em dash read JONES, L. R.
Page 349, line 10, for "tomato, sunflower," read tomato, and sunflower.
Page 349, line 11, delete "and pea"
Page 353, citation 9, for "BARTLETT," read BARTLEY.
Page 474, standard error formula, transpose numerator and denominator.

Vol. 15 No. 1

PLANT PHYSIOLOGY

JANUARY, 1940

RESPONSES OF THE BEAN PLANT TO CALCIUM DEFICIENCY

HUGH GAUCH

(WITH SEVENTEEN FIGURES)

Introduction

Calcium has long been regarded as an essential element for most plants, and many rôles have been assigned to it by numerous investigators. It is, however, beyond the scope of this paper to review all of the literature dealing with calcium, or with each of the many rôles that have been attributed to it. The recent review by MILLER (11) is an excellent source for a general survey of the literature.

Many studies have been made comparing the growth of various plants with and without calcium in the nutrient solution. In many cases, however, the solutions have been chosen arbitrarily without a thorough understanding of the effects produced by various combinations of salts. Experiments with various single salts, two-salt combinations, and various total concentrations of salts were deemed necessary in order to obtain the most favorable plus- and minus-calcium solutions for the growth of the bean plant. The differences between the plants growing in these two solutions are believed to indicate more truly the morphological and chemical alterations that result from a deficiency of calcium in the nutrient solution.

Materials and methods

The plant used in this study was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were obtained from a commercial grower of beans at Marysville, California. In these studies the seeds were planted at a depth of one inch in typical greenhouse flats containing unused white, coarse sand, and germinated in darkness for seven days. The sand was kept moist by additions of tap water (containing approximately 32, 11, 1, 0.1, and 15 p.p.m. of calcium, magnesium, potassium, nitrate, and sulphate, respectively). Bean seeds are very sensitive during germination to an over- or an under-supply of

moisture. Temperature in the dark room fluctuated from 78° to 80° F., and the relative humidity varied from 30 to 50 per cent. The seedlings were from seven to eight days old at the time of transfer to the nutrient solutions (fig. 1).

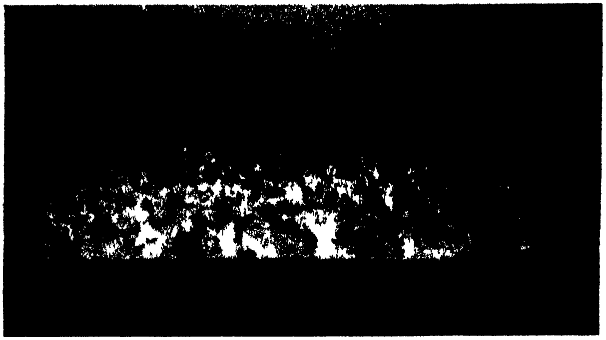


FIG. 1. Seven-day-old bean seedlings as transferred to nutrient solution.

The flats were flooded with tap water to facilitate removal of the seedlings, and after the sand was washed from the roots, the roots were temporarily immersed in distilled water before planting. In each planting, 360 seedlings were accommodated by the 72 one-half gallon glazed crocks, *i.e.*, five plants per crock. The crocks were covered with sheets of cork (paraffined), 6″ × 6″ × ¼″, which served as supports for the plants. Nonabsorbent cotton was pressed lightly into the space between the hypocotyl and the cork support (fig. 4). Early experiments on bean showed that aeration of the cultures was unnecessary for the length of the growth period under study.

As mentioned earlier, many single salts as well as two-salt combinations in various total concentrations were tested; the details of these experiments are reported later. The growth responses—morphological and chemical—are reported in detail for two types of plus- and minus-calcium solutions: (1) those used by NIGHTINGALE, *et al.* (**12**) (designated "solutions A+ and A–"),

TABLE I

COMPOSITION OF THE PLUS- AND MINUS CALCIUM SOLUTIONS

SOLUTION AND SYMBOL*	PARTIAL VOLUME-MOLECULAR CONCENTRATION				
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	KNO_3	$NaNO_3$
Nightingale, *et al.*:					
Plus-calcium (A+)	0.018	0.009	0.009		
Minus-calcium (A–)	..	0.009	0.009	0.026	
For bean:					
Plus-calcium (B+)	0.0075	0.005	0.0005		
Minus-calcium (B–)	...	0.005	0.0005		0.015

* Plus 0.5 p.p.m. of boron, as boric acid, and 10 p.p.m. of iron, as ferric citrate.

and (2) solutions adapted to the bean plant (designated "solutions B+ and B–"). Solutions B+ and B– were prepared on the basis of the results obtained from single-salt and two-salt studies covering a wide range of concentrations (table I).

At the time of harvest, the plants were divided into five fractions, *viz.*, root, hypocotyl, cotyledons, primary leaves, and a residual fraction (first internode plus that portion above the primary leaves). The latter fraction is referred to as "first internode and 'top'."

Physical measurements

Fresh weights of plants, or plant fractions, were determined on a torsion balance sensitive to 0.1 gram. Oven-dry weights were determined on plant material that had been dried at 100° C. in an electric oven for 24 hours, after which it was dried to constant weight in a vacuum oven at 80° C. Oven-dry weights were obtained by use of an analytical balance.

Chemical methods

Total nitrogen.—Total nitrogen was determined by the Gunning method modified to include the nitrogen of nitrates (7).

Ash determinations.—Duplicate samples of the oven-dry material were ashed to constant weight at a dull-red heat in a muffle furnace.

Calcium determinations.—Calcium was determined, with slight modification, according to the method outlined by Lindner and Kirk (6). After ashing, the sample was dissolved in 1:1 hydrochloric acid, and 10 ml. of ammonium oxalate (saturated) plus two drops of brom-cresol-purple, were added. The mixture was heated just to boiling, and dilute ammonium hydroxide was added until the indicator turned purple. After standing overnight for precipitation, the mixture was filtered and the precipitate of calcium oxalate was washed with dilute (10 per cent.) ammonium hydroxide, saturated with calcium oxalate. A special type of sintered-glass filter was used in order that the final titration of the oxalic acid might be carried out in the same Erlenmeyer flask in which the calcium oxalate was precipitated, and hence minimized any loss. The calcium oxalate was dissolved in 2 N sulphuric acid, heated on a hot plate, and 20 ml. of 0.01 N (or 0.1 N, with the larger amounts of calcium) ceric sulphate were added before setting aside to cool. The excess ceric sulphate, that remained after reacting with oxalic acid, was titrated with 0.01 N ferrous ammonium sulphate, using the ortho-phenanthroline-ferrous complex as the indicator.

Experimentation

Single-salt studies

In order that the effect of a single salt alone might be determined, plants were grown in various single-salt solutions over a wide range of concentra-

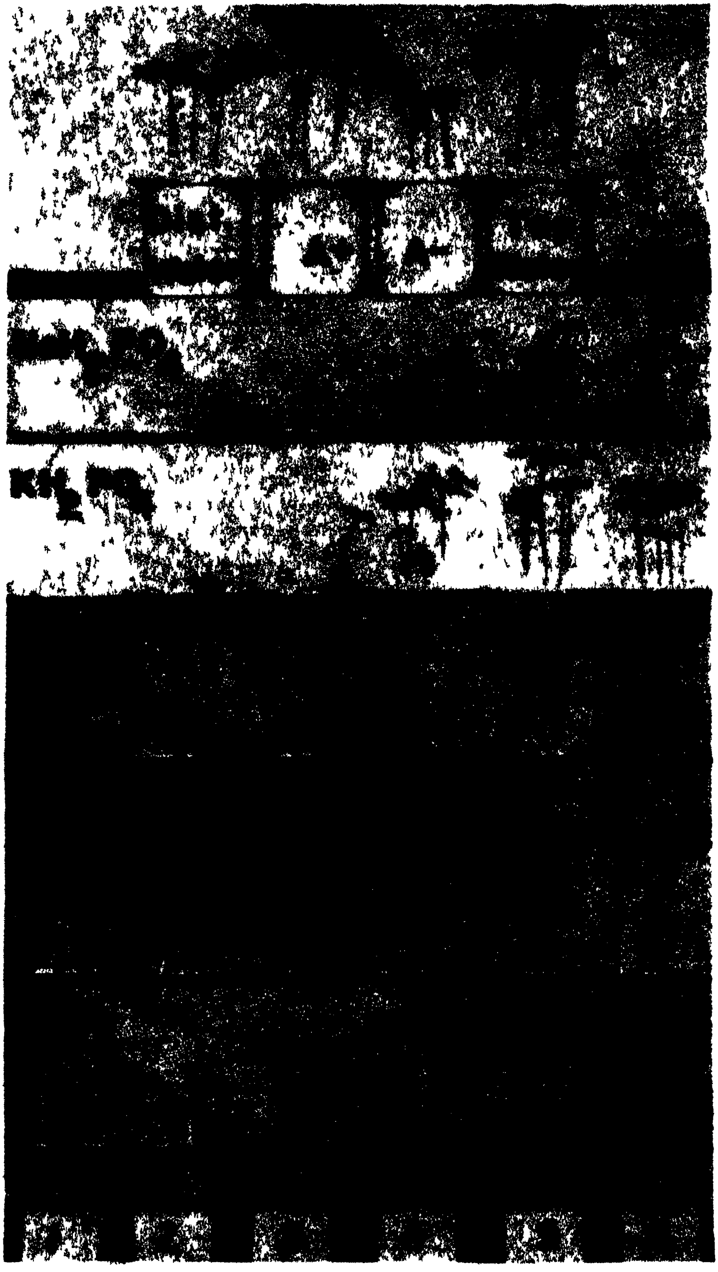


FIG. 2. Bean plants, 9 days after planting into various concentrations of single-salt solutions. Concentrations, vertical rows 1 to 6, are: 0.5, 0.1, 0.05, 0.01, and 0.001 M.

tions, including 0.5, 0.1, 0.05, 0.01, 0.001, and 0.0001 molar. $Ca(NO_3)_2$, $CaCl_2$, $MgSO_4$, $MgCl_2$, KNO_3, KCl, KH_2PO_4, $NaNO_3$, NaCl, NaH_2PO_4, and Na_2SO_4 were used. For all except the two calcium salts, 0.1 and 0.5 M proved to be too concentrated, while the response of plants in the 0.0001 M concentration was essentially like that produced by distilled water (fig. 2). With the exception of Na_2SO_4, which was very toxic, responses in the sodium and potassium salts were much alike. Representative salts of these two cations are shown in figure 2.

High amounts of potassium and sodium produce similar, characteristic symptoms. With an excess of either of these two cations, the hypocotyl becomes more or less water-soaked in appearance and assumes a light tan color.

Little or no root growth resulted in any solutions except those of the calcium salts. With the magnesium salts there was a typical burning of the pulvinus at the base of the primary leaves, and spotting and necrosis of the leaf blades. Moreover, by the second day after planting, the hypocotyls were a very deep, reddish-brown color in the 0.5, 0.1, and 0.05 M concentrations. The coloration first appeared opposite the protoxylem points, and then soon spread throughout the hypocotyl tissue in general.

TWO-SALT COMBINATIONS

After determining the effects produced by a number of single salts, two-salt combinations were studied. The three combinations that were studied dealt with $Ca(NO_3)_2$ and $MgSO_4$, $Ca(NO_3)_2$ and KH_2PO_4, and KH_2PO_4 and $MgSO_4$. Each salt in varying amounts (0.05, 0.5, 2.5, 5, 12.5, and 25 ml. of molar salt per liter of solution) was combined separately with each of the other two salts. This method of combining these salts yields 108 different solutions. Dry weight of the tops, minus cotyledons, was taken as the criterion of growth. If one considers the average growth produced by the various amounts of $MgSO_4$ or KH_2PO_4, when plotted against $Ca(NO_3)_2$, on the abscissa, 5 to 12.5 ml. of molar $Ca(NO_3)_2$ per liter of solution is the optimal amount (fig. 3A). Combining $MgSO_4$ with KH_2PO_4 (fig. 3B), 5 ml. molar KH_2PO_4 per liter is optimal; 2.5 to 5 ml. is the optimal amount of molar KH_2PO_4 when combined with $Ca(NO_3)_2$ (fig. 3B). When KH_2PO_4 is combined with $MgSO_4$ (fig. 3C), 0.05 ml. of molar $MgSO_4$ per liter is suboptimal, 0.5 ml. is optimal, and greater amounts of $MgSO_4$ result in progressively less growth of the plants. Also of interest is the fact that with the optimal amount, or less, of $MgSO_4$ there is no magnesium-burning or toxicity. When $Ca(NO_2)_2$ is combined with $MgSO_4$, 0.05 ml. of molar $MgSO_4$ per liter is also limiting, while 0.5 ml. gives optimal and maximal dry weight of plants. Thus it is shown that when $MgSO_4$ is combined with a calcium salt the higher amounts (above optimum) of $MgSO_4$ can be tolerated by the plants with no decrease in the amount of growth. This is not true for the combination of KH_2PO_4 with $MgSO_4$ (fig. 3C).

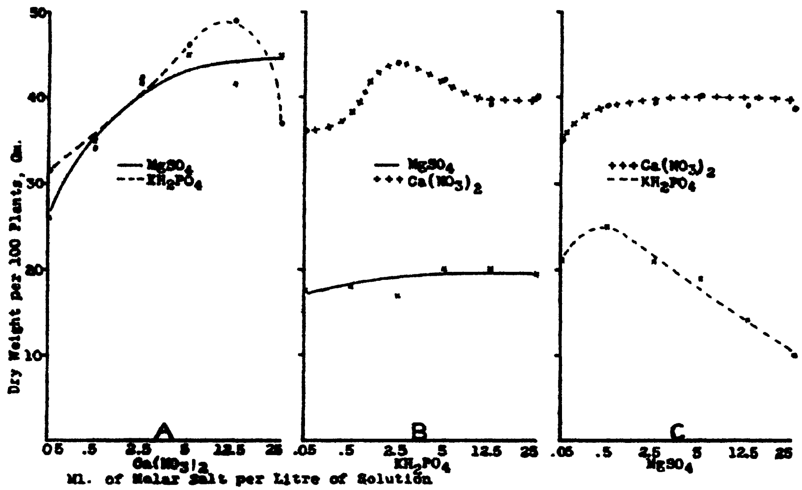


FIG. 3. Growth of bean plants in two-salt solutions. Each curve represents the average growth produced by the various concentrations of a given salt when combined with the salt shown on the abscissa.

On the basis of the results from the single-salt solutions, the two-salt combinations, and the dilution studies (in the following section), the B+ and B− solutions were compounded.

DILUTION STUDIES

The A+ and A− solutions, *i.e.*, 2 atmospheres, and various dilutions of

TABLE II

SIX-DAYS' GROWTH OF BEAN PLANTS IN VARIOUS CONCENTRATIONS OF THE A+ AND A− SOLUTIONS

CONCENTRATION OF SOLUTION	OVEN-DRY WEIGHT PER 100 PLANTS	
	TOPS*	ROOTS
atm.	*gm.*	*gm.*
A+:		
2.0	35.5	4.4
1.0	42.0	5.7
0.1	38.4	5.5
0.01	30.6	5.4
0.001	24.6	3.4
Tap water	30.0	5.3
A−:		
2.0	18.0	0.9
1.0	19.3	1.3
0.1	28.0	2.9
0.01	26.4	3.8
0.001	24.0	3.3
Distilled water	26.6	3.0

* Minus cotyledons.

them (to 1, 0.1, 0.01, and 0.001 atm.) were used, with tap water and distilled water as controls. Dry weights of the tops and roots were taken as the criteria of growth. The results are shown in table II and figure 4.

It is of interest to note that in the A– series the greatest dry weight of tops occurred in the 0.1-atm. concentration. In this concentration the magnesium content is 1/20 of the amount present in the regular A– solution. With a concentration of 0.1 atm. the leaves of the plants did not show magnesium

FIG. 4. Bean plants, 5 days after planting, in various dilutions of the A+ and A– solutions. Concentrations are: 2, 1, 0.1, 0.01, and 0.001 atm., crocks 1 to 5; crock 6 is tap water (above), and distilled water (below).

toxicity. This was evidence additional to that obtained in the single- and two-salt studies, suggesting that the magnesium content of a minus-calcium solution should be approximately 0.5 ml. of molar $MgSO_4$ per liter of solution. This amount of $MgSO_4$ was used, therefore, in the B+ and B– solutions as applied to bean plants during the stage of development under study. Other stages of development might very likely require different amounts of magnesium and other ions, as illustrated for wheat by McCALL and RICHARDS (8).

INTERRELATION OF CALCIUM AND MAGNESIUM

Inasmuch as it is impossible to designate a single value for the Ca/Mg ratio that will prove "best" in all combinations or all concentrations of salts, it seems preferable to speak of the interrelation of calcium and magnesium, potassium, or other ions. The word "ratio" is convenient, however, for the purpose of expressing the relative amounts of one ion to another, and it will be used in that sense only.

In the present study of the interrelation of calcium and magnesium, KH_2PO_4 and KNO_3 were kept constant at 9 and 26 ml. of molar salt per liter, respectively, while $Ca(NO_3)_2$ and $MgSO_4$ were varied to produce Mg/Ca

ratios of 1, 10, 20, 30, 40, and 50. In one series there was 0.18 ml. of molar $Ca(NO_3)_2$ per liter of solution, and the $MgSO_4$ was varied to produce the above-stated ratios. In another series $Ca(NO_3)_2$ was at 1.8 ml.; in still another, at 18 ml. of molar salt per liter of solution, the $MgSO_4$ being varied accordingly. Thus, although the same set of ratios existed in each of the three series, the total concentration of the series varied, as did also the concentration of the members within a series.

A given ratio of Mg/Ca is not the only factor that will determine plant response, as is illustrated in figure 5. Most of the plants in row C were killed by the high concentrations of salts. In row B the various heights of plants in the different crocks are apparently related to the various concentrations There was little or no magnesium injury in rows A and B with Mg Ca ratios of 10 or less. This was more obvious in the case of row B than in A, showing that the effect of ratios may be altered by the total concentration of salts. According to OSTERHOUT (13), growth in concentrated (0.1 M) solutions furnishes a much better criterion of antagonism than growth in dilute solutions; at great dilution about the same amount of growth is obtained regardless of the proportions of the two salts. For wheat, TOTTINGHAM (17) and SHIVE

FIG. 5. Responses [illegible] plant to the same series of Mg/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B and C contain 0.18, 1.8, and 18 ml. of molar $Ca(NO_3)_2$ per liter, respectively.

(15) report that the Mg/Ca ratio must be 2, or less, to avoid magnesium injury. It is of interest that a Mg/Ca ratio of 1.0 was not as favorable for the growth of bean plants as the higher ratios. TOTTINGHAM (17) has reported that high concentrations of $Ca(NO_3)_2$, in a series of solutions with two per cent. (8.15 atm.) concentrations, produced characteristic and serious injury to the tops of wheat plants.

TRELEASE and TRELEASE (18) state that magnesium injury to wheat, while mainly controlled by the Mg/Ca ratio, appears to be influenced by the proportions and concentrations of other materials present in the culture solution, as well as by the total concentration of the solution and possibly also by climatic conditions. They also report that, between the limits of one and 50 of this ratio, the dry weight of tops of wheat plants is inversely proportional to the log of the Mg/Ca ratio. TRUE and BARTLETT (19) found that absorption by pea roots is greatest in equimolecular proportions of calcium and magnesium nitrates, but that root growth is equally good in any solution which does not contain less than one molecule of calcium to nine of magnesium. In the more dilute solutions, an even lower ratio of Ca/Mg, 1:99, overcame the effects of magnesium alone. GARNER, *et al.* (5), state that it is difficult to distinguish between magnesium toxicity symptoms and minus-calcium symptoms. They found that, in the case of tobacco, there must be present in the leaf 0.25 per cent. of magnesium and 1.0 per cent. of calcium to prevent deficiency symptoms of these cations.

BARBIER (1), working with maize, oats, onion, spinach, and tomato reported that the uptake of an element is a function of its concentration, and concluded that antagonism is relatively unimportant and should not be exaggerated. Similarly, in studies with corn, BECKENBACH, WADLEIGH, and SHIVE (2) observed that none of the three ratios—Ca/Mg, K/Mg, or K/Ca—showed any significance.

This study indicates that there is no well-defined Ca/Mg "ratio" that is optimal for growth over a range of total concentrations of salts, and that it is a matter of interrelationships among calcium, magnesium, and other ions of the solution.

INTERRELATIONSHIP OF CALCIUM AND POTASSIUM

In another series, similar to the Mg/Ca series, calcium and potassium were varied, while $MgSO_4$ was constant at 9 ml. of molar salt per liter of solution. Calcium nitrate was used at three levels of nutrition, just as in the previous experiment, and the two potassium salts (KNO_3 and KH_2PO_4) were varied to produce K/Ca ratios of 200, 150, 100, 50, 10, and 1. Within 7 days, the appearance of the plants was as shown in figure 6. Rows B and C show growth responses that were apparently associated with concentration, *i.e.*, total concentrations become less from left to right. There was no magnesium burning

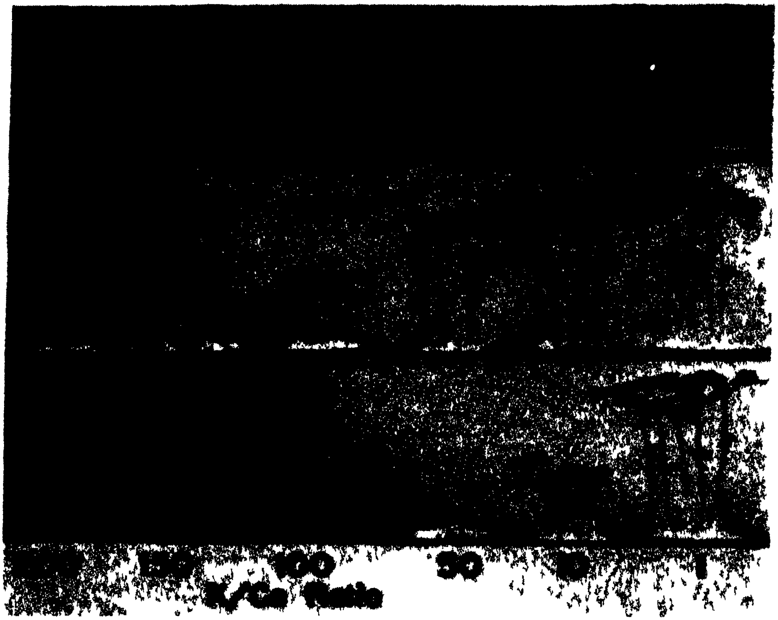


FIG. 6. Responses of the bean plant to the same series of K/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B, and C, contain 0.18, 1.8, and 18ml. of molar $Ca(NO_3)_2$ per liter, respectively.

in either row B or C. In row B there was no burning, and the ratio of Mg/Ca was only five. In row A the plants were of similar heights and growth-form, but all of the plants in this row were severely burned owing to the high Mg/Ca ratio of fifty.

According to McCALLA and WOODFORD (9), limiting the amount of one element in a nutrient brings about an increase in absorption of another ion of the same sign, or a decrease in absorption of all ions of the opposite sign. Limitation of potassium is accompanied by an increase in the absorption of both calcium and magnesium. Limitation of calcium results in a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater.

Thus it seems that calcium plays an important rôle within the cells and in the so-called physiological balance of nutrient solutions by overcoming within certain limits, the toxic effects of too high a concentration of any other cation. This interrelation of the various cations is not a static condition it is dynamic and continually varying—depending upon the relative concentrations of the cations present. These interrelations of cations are presumably effective in part through their action on the colloidal properties of protoplasm.

COMPARISON OF THE A+ AND A– SOLUTIONS WITH THE B+ AND B– SOLUTIONS

Calcium deficiency affects the terminal bud, leaves, and roots of the bean plant in much the same way as reported for tobacco by MCMURTREY (10).

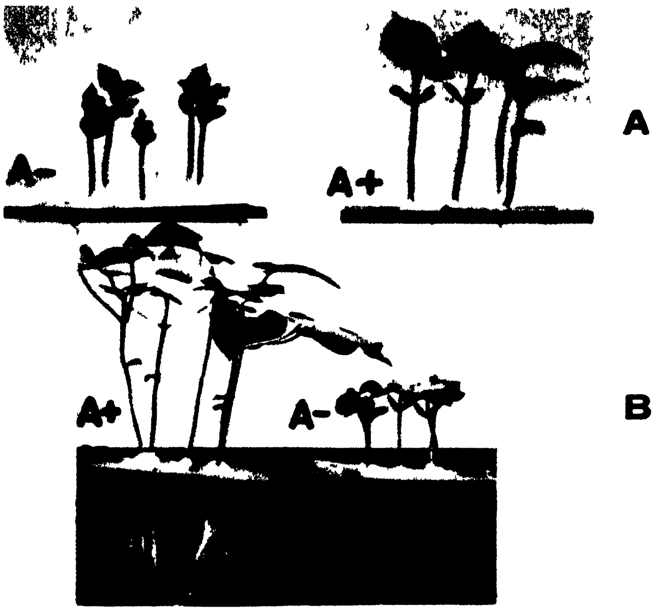


FIG. 7. Growth of bean plants in the A+ and A– solutions. A, three, and B, seven days after planting

Plants growing in the A– solution (fig. 7) are short (stunted) with small, wrinkled leaves. They die within six to eight days from the time of transfer. The dry weight (fig. 9) and total nitrogen (fig. 13) do not increase from the second to the sixth day. Ash constituents (fig. 10) continue to enter these plants, but nitrates do not. This is in accordance with the findings of NIGHTINGALE, *et al.* (12), for tomato. The calcium content (fig. 14) increases slightly and then it decreases. The fact that the roots of these plants contain relatively large amounts of calcium indicates that the calcium, although entering in very small amounts, is bound in the roots In general the dry weight (fig. 11), ash (fig. 12), nitrogen (fig. 15), and calcium (fig. 16) decrease only slightly in the cotyledons of plants growing in the A– solution.

On the other hand, in the B– solution (fig. 8) the dry weight (fig. 9), ash (fig. 10), and total nitrogen of the plants (fig. 13) increase markedly until after the eighth day. The plants live for two weeks or more from the time of transfer, are taller, have larger leaves, and wrinkling, or necrosis, of the

FIG. 8. Growth of bean plants in the B+ and B– solutions. A, four, B, eight, and C, twelve days after planting.

leaves is less pronounced or nearly absent. Ash and total nitrogen increase at approximately the same rate. Despite the fact that the B– solution is a minus-calcium solution, nitrates are absorbed to as great an extent as are the ash constituents. This is in contradiction to the statement by NIGHTIN-

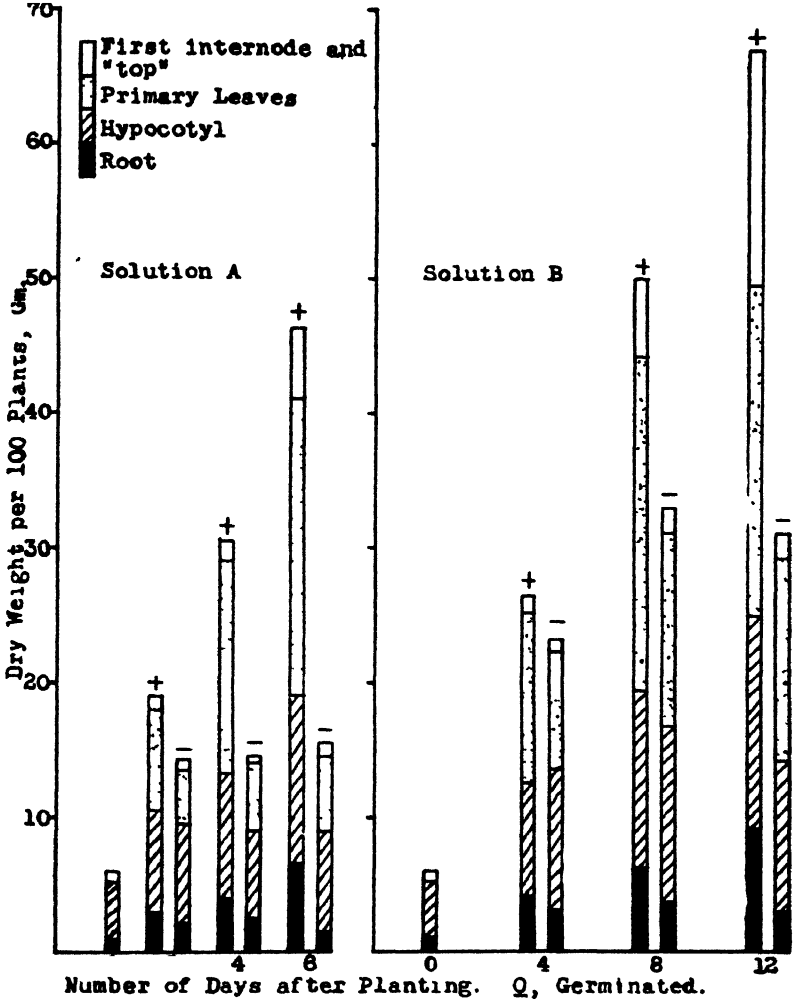


FIG. 9. Dry weight of bean plants.

GALE, *et al.* (**12**), that minus-calcium plants cannot absorb nitrates. It is true that they do not do so in the A– solution, but they do in the B– solution. It seems likely, therefore, that the composition of the A– solution is such that the unbalance in the absence of calcium is responsible for the nonabsorption of nitrates. As mentioned earlier in this paper, the amount of magnesium

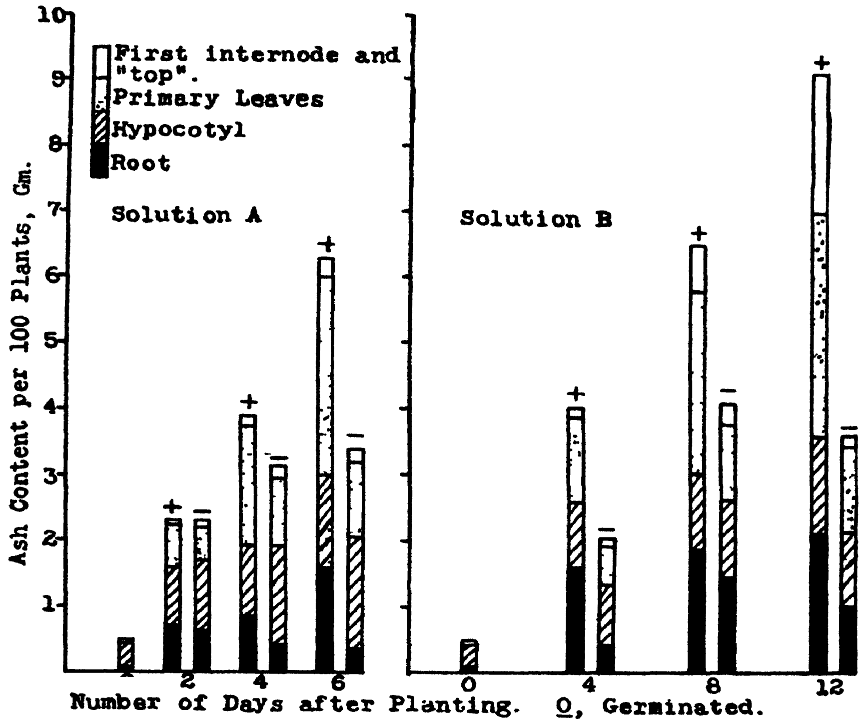


FIG. 10. Ash content of bean plants.

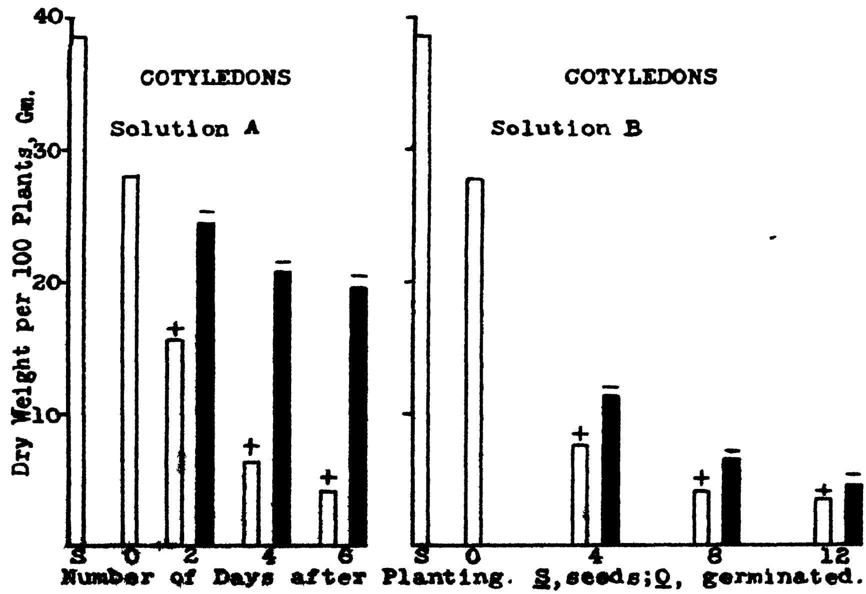


FIG. 11. Dry weight of cotyledons of bean plants.

appears to be one of the main disturbing factors in the A– solution. Inasmuch as the nitrogen content of plants in the B– solution increased markedly, while the calcium content remained more or less constant and low, there is apparently not necessarily a close relationship between calcium and nitrogen contents, as proposed by PARKER and TRUOG (14). There may be a correlation, however, between the utilization of nitrogen and the content of calcium. The latter relationship was not determined in this study, but ERMAKOV (4) and BURRELL (3) have reported that, associated with calcium deficiency, there is an accumulation of nitrates in leaves owing to the fact that nitric nitrogen is not assimilated and converted to organic forms of nitrogen.

In the B– solution, the dry weight (fig. 11), ash (fig. 12), and nitrogen (fig. 15) of the cotyledons decrease to about the same amounts and at the same rate as in the plants of the B+ solution. The calcium content (fig. 16) of cotyledons increases in plants growing in the plus-calcium solutions, and tends to remain more or less constant in the A– or B– solutions. In the absence of sufficient calcium in the nutrient solution, the reserve calcium of the cotyledons is apparently unavailable.

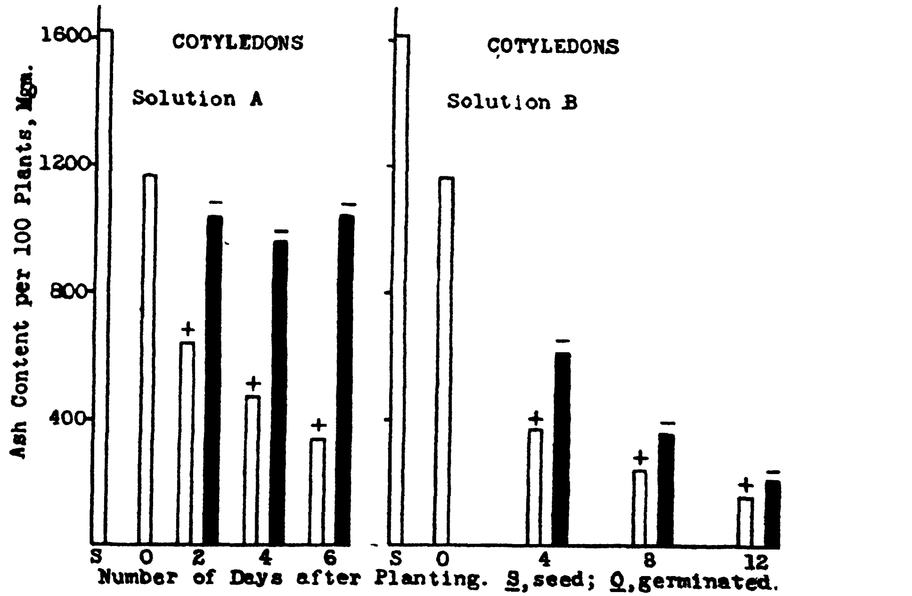


FIG. 12. Ash content of cotyledons of bean plants.

The hypocotyls of plants growing in the A– solution contain more ash than the hypocotyls of plants in the A+ solution, as shown in figure 10. On the other hand, in the B– solution in which nitrates are readily absorbed, the hypocotyls of plants growing in that solution contain more nitrogen (fig. 13) than the hypocotyls of plants in the B+ solution. The reason for this

directional shift of ash and nitrogen to the hypocotyls is unknown, but it is of interest that a similar shift of such materials occurs when the cut surfaces of bean hypocotyls are treated with indoleacetic acid, as reported by STUART

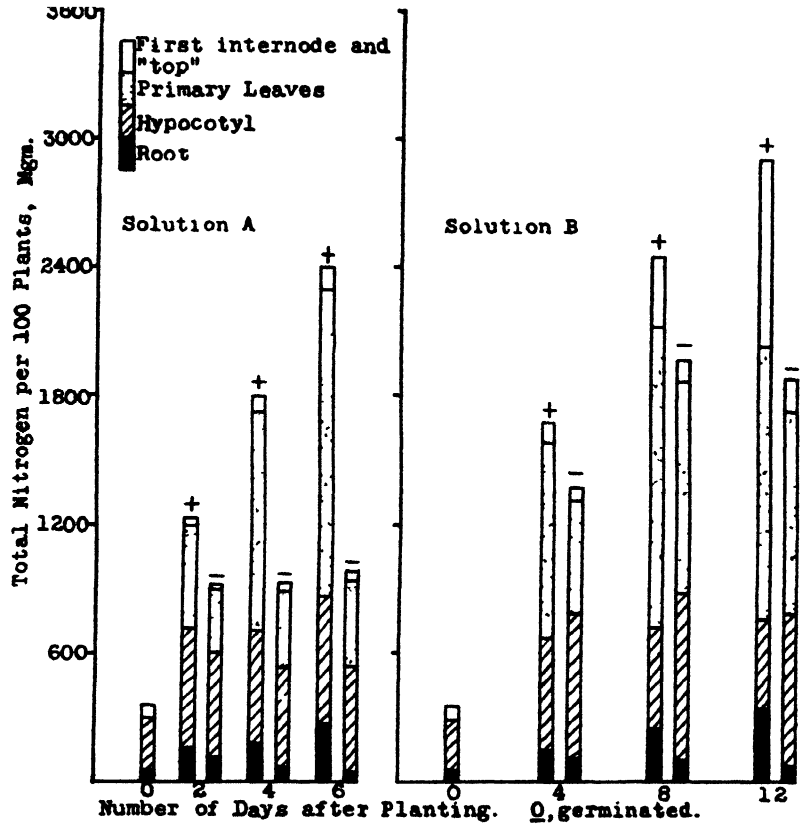


FIG. 13. Total nitrogen content of bean plants.

(16). In the response of the bean plant to a deficiency of calcium, including the degeneration of the original root system, there is a tendency toward the production of adventitious roots, especially near the base of the hypocotyl. Such roots do not reach more than one or two millimeters in length.

GROWTH RESPONSES FOLLOWING TRANSFER OF PLANTS BOTH FROM THE B+ TO THE B– SOLUTION AND FROM THE B– TO THE B+ SOLUTION

Each day, for 12 days after planting, plants were transferred from the B– to the B+ solution; plants were similarly transferred from the B+ to the B– solution. As shown in figure 17, the earlier that B– plants were placed on B+ nutrient, the more completely did they resemble B+ plants, and the better was their recovery in general. If transferred between the twelfth and

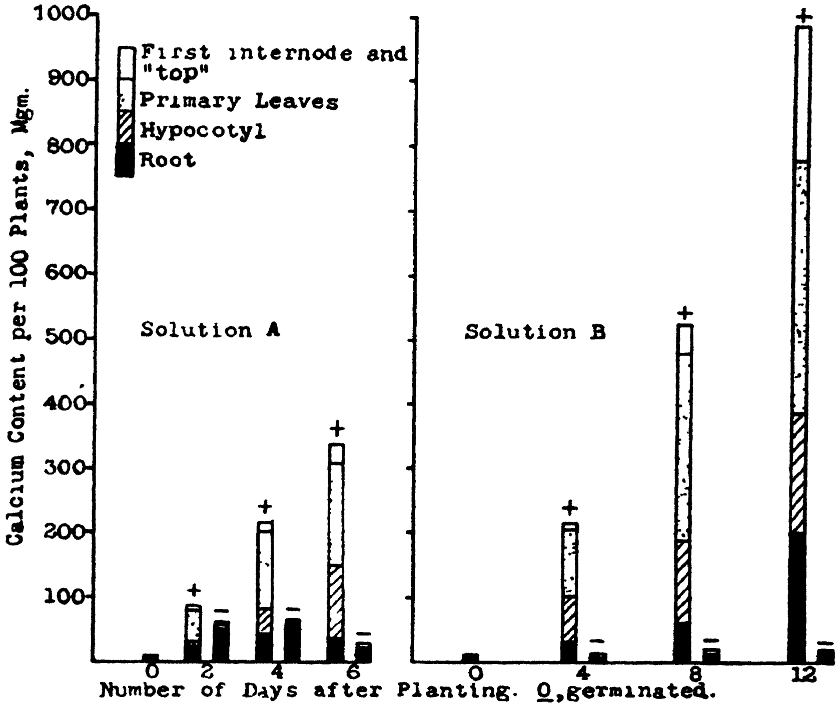


FIG. 14. Calcium content of bean plants.

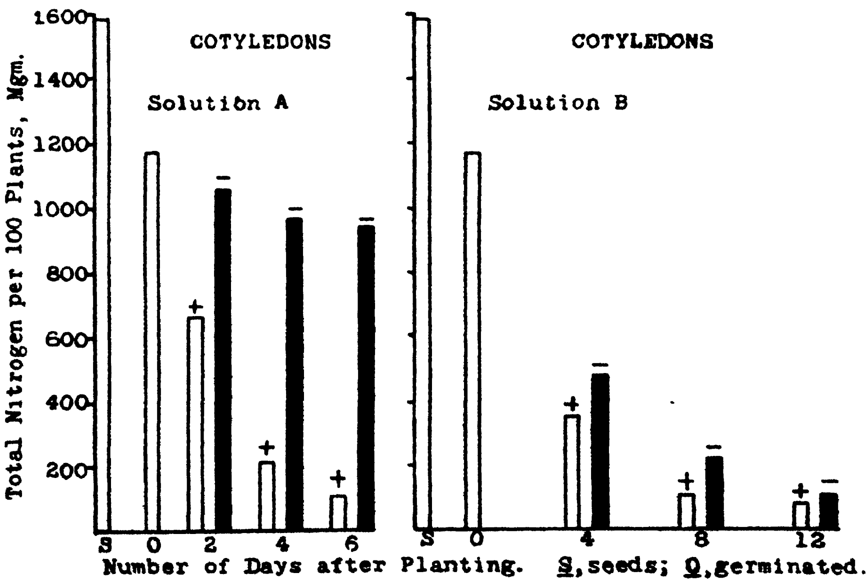


FIG. 15. Total nitrogen content of cotyledons of bean plants.

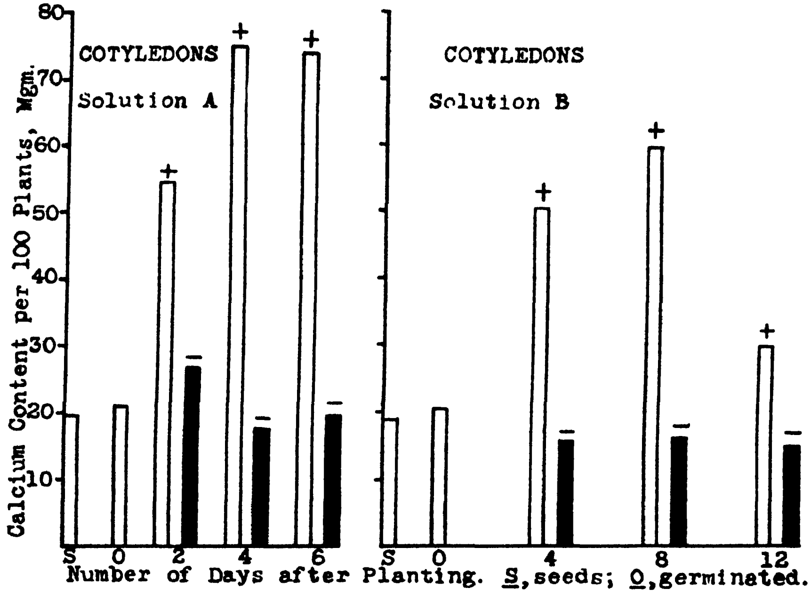


FIG. 16. Calcium content of cotyledons of bean plants.

fourteenth days, the axillary buds of the primary leaves finally grew out, but the terminal bud was dead, as judged from its appearance and from the fact that it did not grow. It seems probable that the terminal bud was originally the most active of the three buds, and that the absence of calcium affected its metabolism first, causing its death before that of the axillary buds. The original roots were dead on the B– plants transferred, after six days, but new adventitious roots arose from the bases of the hypocotyls up to and including at least the twelfth day.

FIG. 17. Response of bean plants after transfer from B– to B+, and from B+ to B– solution. Photograph taken on 13th day after day of planting.

When B+ plants were transferred to B– solution at similar intervals, root growth soon ceased, especially in those B+ plants transferred early into B– solution. Within 10 to 12 days after the second- and fourth-day transfers had been made, the petioles of the primary leaves became weak and the leaves pendant (fig. 17). The cause of this response is unknown, but it approximated the symptoms of high potassium or high sodium. During the first four days after planting, there was a large increase in the amount of ash (fig. 10). In the absence of calcium, apparently relatively greater amounts of other cations are absorbed. With limitation of calcium, McCALLA and WOODFORD (9) observed a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater. In the B– solution, the total magnesium content is low, but, in this instance, relatively greater amounts of sodium may have entered; the latter element acts, within the plant, much the same as does potassium.

Summary

1. Responses of the bean plant to various concentrations of single salts are reported.

2. From the results obtained in the single-salt studies, various two-salt combinations were made, and the responses of the plants growing therein are reported.

3. On the basis of those and other studies, plus- and minus-calcium solutions (solutions B+ and B–) especially adapted to bean were devised in which there is no apparent excess or deficiency of essential ions other than calcium.

4. The responses of bean plants to two types of solutions were studied, *viz.*, (a) NIGHTINGALE'S, *et al.*, plus- and minus-calcium solutions (solutions A+ and A–), and (b) plus- and minus-calcium solutions adapted to bean (solutions B+ and B–).

5. Bean plants growing in the A– solution continue to absorb ash constituents, but they do not absorb nitrates. This is in accordance with the findings of NIGHTINGALE, *et al.*, for tomato.

6. Plants growing in the B– solution do, however, absorb appreciable quantities of nitrates, and the increases in dry weight, total nitrogen, and ash of these plants are parallel.

7. Dry weight, total nitrogen, and ash of the cotyledons from plants growing in the B– solution decrease markedly in amount, while those fractions decrease only very slightly when the plants are grown in the A– solution.

8. Bean plants, with cotyledons intact, live approximately six days in the A– solution, and twelve days in the B– solution.

9. There appear to be no ratios, *e.g.*, Ca/Mg or Ca/K, that are optimal for growth, over a range of concentrations of salts.

The writer is indebted to members of the Department of Botany of the University of Chicago for helpful suggestions made during the course of this study.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BARBIER, G. The mineral nutrition of the plant as a function of the chemical composition of its medium. Ann. Agron. **6**: 568–586. 1936.
2. BECKENBACH, J. R., WADLEIGH, C. H., and SHIVE, J. W. Nutrition studies with corn: 1. A statistical interpretation of the nutrient-ion effect upon growth in artificial culture. Soil Sci. **41**: 469–489. 1936.
3. BURRELL, R. C. Effect of certain deficiencies on nitrogen metabolism of plants. Bot. Gaz. **82**: 320–328. 1926.
4. ERMAKOV, V. P. Relation of the salts of calcium to the assimilation of nitrate nitrogen by green plants. Univ. Izv. (Kiev) **48**: 1–68; abs. in Zhur. Opuitn. Agron. (Russ. Jour. Expt. Landw.) **10**: 394–396. 1908.
5. GARNER, W. W., MCMURTREY, J. E. JR., BOWLING, J. D. JR., and MOSS, E. G. Magnesium and calcium requirements of the tobacco crop. Jour. Agr. Res. **40**: 145–168. 1930.
6. LINDNER, R., and KIRK, P. L. Quantitative drop analysis. VII. Further investigations on the determination of calcium. Mikrochemie **22**: 291–300. 1937.
7. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill Book Co. New York, 1937.
8. MCCALL, A. G., and RICHARDS, P. E. Mineral food requirements of the wheat plant at different stages of its development. Jour. Amer. Soc. Agron. **10**: 127–134. 1918.
9. MCCALLA, A. G., and WOODFORD, E. K. Effects of a limiting element on the absorption of individual elements and on the anion: cation balance in wheat. Plant Physiol. **13**: 695–712. 1938.
10. MCMURTREY, J. E. JR. Distinctive effects of the deficiency of certain essential elements on the growth of tobacco plants in solution cultures. U. S. Dept. Agr. Tech. Bull. 340. 1933.
11. MILLER, E. C. Plant physiology. 2nd ed. McGraw-Hill Book Co. New York, 1938.
12. NIGHTINGALE, G. T., ADDOMS, R. M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
13. OSTERHOUT, W. J. V. The forms of antagonism curves as affected by concentration. Bot. Gaz. **58**: 367–371. 1914.

14. PARKER, F. W., and TRUOG, E. The relation between the calcium and the nitrogen content of plants and the function of calcium. Soil Sci. **10**: 49–56. 1920.

15. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.

16. STUART, N. W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. Bot. Gaz. **100**: 298–311. 1938.

17. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133–245. 1914.

18. TRELEASE, S. F., and TRELEASE, HELEN M. Magnesium injury of wheat. Bull. Torrey Bot. Club **58**: 127–148. 1931.

19. TRUE, R. H., and BARTLETT, H. H. Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. I. Concentration relations of dilute solutions of calcium and magnesium nitrates to pea roots. U. S. Dept. Agr. Bur. Plant Ind. Bull. 231. 1912.

METABOLIC PROCESSES OF POTATO DISCS UNDER CONDITIONS CONDUCIVE TO SALT ACCUMULATION[1]

F. C. STEWARD AND G. PRESTON

(WITH NINE FIGURES)

Introduction

Modern investigations (**11, 34**) show that vital activity plays an essential part in salt accumulation by plants. The ability to accumulate[2] demands properties peculiar to cells which are still able to grow and the rate at which these are utilized is causally related to the intensity of aerobic respiration. Salt accumulation is not merely the result of equilibria at membrane surfaces but demands some "dynamic machinery" in cells—to use a happy phrase which received prominence in a recent symposium. A similar concept is implied by the oft repeated assertion that during salt accumulation work is done and energy exchanges are involved. The identification of the "dynamic machinery" and the mechanism by which the energy stored in substances of high energy content is ultimately utilized in salt accumulation is one of the most challenging problems of modern plant physiology.

The earlier work, done on Nitella, Elodea, discs cut from storage organs, and roots, focussed attention upon those biochemical processes in the accumulating cells which involve carbohydrates and suggested that these represent an important part of the machinery of accumulation. In a brief review of available evidence (**33**) it was concluded that the biochemistry of salt absorption represented a virgin field for investigation and this inspired much of the work to be described in this and ensuing papers.

The relationship between salt accumulation and metabolism has been most investigated with reference to respiration as measured by carbon dioxide output. The conclusion to be drawn (**11, 33, 34**) is that the relation between salt uptake and respiration is not a simple and direct one; it concerns the aerobic phases of the respiratory process but it is the oxidation which these involve, rather than the production of carbon dioxide *per se,* which is causally concerned in salt accumulation. The ultimate connection between respiration and salt accumulation must therefore involve some aspect of the oxidative breakdown of sugar—the ultimate source of carbon substrates—other than the mere evolution of the carbon dioxide which is finally produced. The energy thus released must be involved; not only because it supplies the relatively small amount of energy necessary for the actual process of salt uptake, (a process which tends to increase the free

[1] This is the first of a group of papers on the biochemistry of salt absorption.

[2] Especially that most general type of accumulation which involves the simultaneous uptake of anion and cation and which has been termed "primary absorption." Certain absorption processes in which metabolism is not involved are not in question here.

energy of the system concerned) but also because it maintains the cells at that general level of metabolic activity which is an invariable characteristic of systems capable of a high degree of salt accumulation. Hence the biochemistry of salt accumulation entails an account of the fate of the carbohydrates metabolized during salt accumulation—an account which must ultimately be tested by a balance sheet of carbohydrates and their energy values. This aspect will be considered in a subsequent paper (**36**).

The relation between salt uptake and metabolism cannot, however, be restricted even to the processes of carbohydrate metabolism for it demands also a general picture of all the metabolic processes of cells engaged in salt accumulation. Only by the effect of the variables which determine accumulation upon these metabolic processes can those which are causally connected with salt uptake be recognized. It has been emphasized (**37**) that the conditions conducive to salt accumulation should also cause not only an enhanced respiration (oxygen uptake and carbon dioxide production), but also protein synthesis at the expense of soluble nitrogen reserves of the storage tissue. These processes should also be accompanied by a considerable evolution of heat; in fact, by all those processes which eventually lead to cell division and are involved in a recrudescence of growth in cells which were previously dormant in the storage organ. At that time much had to be inferred from evidence cited from other investigations as actual quantitative measurements of some of the metabolic processes described had not then been made.

The new evidence establishes the reality of some of the metabolic processes of thin, aerated potato discs which was hitherto presumed upon less direct evidence. This paper indicates the scope of the survey of metabolism which the general problem demands and deals with methods suitable for this type of investigation. Justification for the experiments to be described does not rest alone upon the more complete account they give of the metabolites of cells engaged in salt accumulation; they may also be scrutinized from another and still more general standpoint. The true nature of the relation between respiration and concomitant metabolic processes is as obscure as that which respiration bears to salt accumulation. Since the experiments to be described in this and subsequent papers record the respiration and metabolism of tissue subject to rigorous control, the data are relevant to the more general problem. The ultimate objective is a synthesis of results into a scheme which explains the mutual relations of all those vital processes which are dependent upon respiration for energy. The experiments to be described in this and following papers contribute to this end.

For the problem as thus envisaged the cut discs of storage tissue used in the earlier work have special advantages. The experimental technique (**31**) devised for the investigation of respiration and salt uptake is also necessary for the investigation of metabolism since the variables thus controlled (oxygen supply, temperature, and disc thickness) also control metabolism. The

amount of tissue used in each culture vessel (not exceeding 60 standard discs of 0.75 mm. thickness and a total weight of 45 gm. or its equivalent surface) is adequate for detailed biochemical examination and, being random sampled from a large batch of discs, the variability between replicate cultures can be reduced until it becomes negligible for most purposes. The general effect of oxygen concentration, temperature, and disc thickness upon respiration and salt uptake of potato discs is known and this information may be used when arbitrary values must be assigned to these variables. Previous work has localized the most active cells which contribute the bulk of the total respiration of the discs to a thin, hollow, cylindrical shell of tissue—the dimensions of which are known for standard conditions—which is situated at the surface of the disc. The technique which has evaluated the respiration rate, or the salt accumulation, of cells in given situations within the disc could also be applied to other metabolic processes. Hence the physiological behavior of thin discs of storage tissue need no longer be interpreted on the mistaken assumption that all of the cells of the disc are identical; on the contrary this type of experimental material has its greatest utility when it is recognized that the degree of activity in the cells is determined by their position within the disc. Thus the correct emphasis may be given to the very active cells which constitute that thin shell of tissue in which the properties of growth and active metabolism reside. Relative to the larger problem which involves the relation between respiration and other metabolic processes there is special reason for selecting experimental material in which cells retain the capacity for growth and cell division and also environmental conditions which permit these properties to be exercised. Senescent cells which have lost the properties in question clearly lack some fundamental metabolic property and exhibit a relationship between respiration and oxygen concentration which differs from that encountered in cells which are still able to grow. For example, contrast the effects of oxygen concentration on the respiration of potato discs (**32**), carrot and artichoke (**35**), potato roots (**35**), barley roots (**11**), and various seedlings (**15, 16**) with the effects of oxygen on the respiration of apples (**21**), tomato fruits (**10**), etc. The relationship of respiration to metabolism, therefore, may be different in kind in senescent and growing cells and there is every reason to believe that the latter constitute the most general case for the investigation of such problems.

Experimental methods and results

Composition of potato discs and basis for the expression of analytical results

The basis for the calculation of the results of analyses which portray the course of metabolism should be some quantity which is not subject to change.

Neither the final fresh nor dry weight of the rinsed, surface-dried discs fulfil this requirement. The initial fresh weight of a known number of standard discs is free from objection since the initial composition of replicate batches of discs random sampled from a large stock can be accurately reproduced. Variation in the fresh weight of replicate batches of standard potato discs, whether cut at the same time or not, is small if the stock of tubers is uniform (table I).

TABLE I

MEAN FRESH WEIGHT OF 50 DISCS

No. of replicates	Mean fresh weight	Standard deviation	Standard deviation as percentage of mean
	gm.	*gm.*	%
8	38.5	0.49	1.27
8	38.2	0.32	0.85

The uniformity in size and fresh weight of the discs cut and then washed 24 hours in running tap water does not conceal a much greater disparity in their composition as shown by the data of table II.

TABLE II

THE DRY WEIGHT AND CARBON CONTENT OF POTATO DISCS

No. of replicates	Fresh wt.	Dry wt.			Carbon content		
	Mean	Mean	Standard deviation* as percentage of mean	As percentage of fresh wt.	Mean	Standard deviation* as percentage of mean	As percentage of fresh wt.
	gm.	*gm.*	%	%	*gm.*	*gm.*	%
4	27.8	4.22	1.73	15.1	1.62	5.57	38.6

* Standard deviation of the mean as a percentage of the mean = ½ this amount.

Table III shows the total sugar and nitrogen content of standard, washed discs cut from the same stock at various times during one month of the rest period. It will be seen that both the differences between parallel samples (series C and D) and between the different time series (series A, B, C, D) are small relative to the order of the effects to be described later. Where comparisons between experiments not run concurrently are made in this work the experiments were carried out within the shortest possible time to eliminate effects due to changes during storage.

Even where known varietal differences and great disparity in the soil

TABLE III

SUGAR AND NITROGEN CONTENT OF STANDARD DISCS

SERIES	SUGAR PER GRAM FRESH WEIGHT	TOTAL NITROGEN PER GRAM FRESH WEIGHT	FRESH WEIGHT PER DISC
	mg.	*mg.*	*gm.*
A	3.53	2.06	7.43
B	2.74	2.05	7.56
C	3.15	2.05	7.91
	3.24	2.08	7.90
D	2.99	2.06	7.86
	3.20	2.05	7.94

TABLE IV

COMPOSITION OF DIFFERENT STOCKS OF POTATO DISCS

STOCK	DRY WEIGHT PER 45 GRAMS FRESH WEIGHT	STARCH PER 45 GRAMS FRESH WEIGHT	SUGAR PER 45 GRAMS FRESH WEIGHT	PROTEIN N PER 45 GRAMS FRESH WEIGHT	SOLUBLE N PER 45 GRAMS FRESH WEIGHT
	gm.	*gm.*	*gm.*	*mg.*	*mg.*
1934	6.40	4.28	0.22	37.8	59.0
1935	(a)5.78	4.04	0.16	34.6	56.7
	(b)5.74	3.79	0.12	33.2	59.4
	(c)5.43	4.03	0.14	30.6	61.6

conditions are involved the principal organic constituents of standard potato discs are remarkably constant. This is well shown by the analysis of the two principal stocks of tubers used in the work to be described in this and a later paper. The one (1934, experiments at Berkeley, California) was a variety grown in the Delta soils of California which are rich in salts. The other (1935) represents King Edward tubers grown in Lincolnshire, England; samples A, B, and C were taken at different times during the storage period when experiments were made. Despite the evident uniformity of the composition of potato tubers the comparisons were mainly made between parallel treatments applied to discs random sampled from a uniform batch cut at one time.

METABOLIC PROCESSES INVESTIGATED AND METHODS USED

The processes chosen for investigation and the technique adopted are outlined below.

CHANGE IN FRESH WEIGHT.—As in earlier work, this very valuable index of the behavior of the tissue was obtained by weighing the discs after they

were rinsed, and the surface dried twice with blotting paper. When the final sample was subdivided for analysis the fresh weight of the various aliquot parts was recorded.

Change in dry weight.—Dried samples were used for some of the determinations (*e.g.*, calorific value). The dry weights were determined as follows. The fresh tissue was heated *in vacuo* for one hour at 100° C. to destroy enzymes. Constant weights were obtained after 36 hours drying *in vacuo* at 60° C. The dried ground tissue was always redried *in vacuo* at 60° C. before aliquot parts were withdrawn for analysis.

Respiration.—Methods by which the respiration of potato discs immersed in aerated salt solutions can be measured have been fully described (**31**). Whenever the total carbon dioxide—in contrast to the average rate during a brief period—was required, the residual external solution was acidified, aspirated, and the carbon dioxide yield determined. This correction attains importance in nitrate and calcium solutions.

When effects of salts on respiration are involved the interaction between the salt effect and time is often of importance. Hence methods which yield data at shorter time periods were desired and for this purpose the respiration determination was modified as follows.

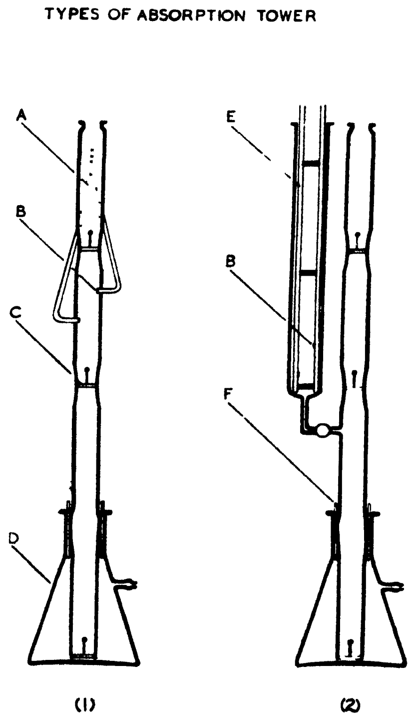


Fig. 1. Types of absorption tower for use with conductivity methods.

Into the modified Reiset absorption towers (fig. 1), of the type previously described (**31**) platinized-platinum electrodes [B, (1) fig. 1] with an area of 0.18 cm.[2] were inserted at a level between the two upper perforated silver discs. To increase electrical resistance in contact with a maximum concentration of 1.0 M caustic soda the electrodes were placed 2.0 cm. apart horizontally and 3.0 cm. apart in the vertical direction as in the method of NEWTON (**20**). External contact was made by means of tubes containing mercury [A, (1) fig. 1]. The gas stream was stopped during readings and the contents of the tower previously mixed by raising and lowering the liquid. It is sometimes convenient to insert before the tower a T-piece and a capillary resistance which can be opened at will, by a screw clip, to air. In this way the gas stream may be temporarily diverted and by operation of screw clips the absorptión liquid can be rapidly raised and lowered. Others have shown (**4, 20**) that over a wide range the decrease in conductivity of a caustic alkali solution bears a linear relation to the carbon dioxide absorbed. The towers in question were calibrated for a fixed volume of alkali by carbon dioxide generated from known amounts of bicarbonate solution. Specimen curves for two such towers[3] are shown (fig. 2). The equation to the line shown was

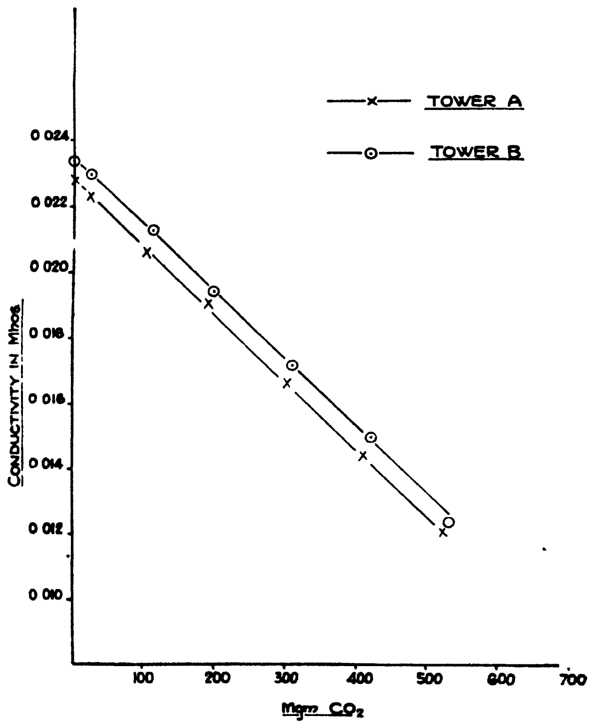


FIG. 2. Calibration curves of absorption towers.

[3] Charged with 50 ml. of MNaOH plus 100 ml. of water.

found by the method of least squares. For a constant volume of absorbing liquid (150 ml.), the constant of slope in the equation is characteristic of the tower and is unaffected within a narrow range, by the total concentration of alkali. Respiration rates were most conveniently expressed as mg. carbon dioxide per gm. initial fresh weight per hour.

Figure 1, (2) shows another modification which has the advantage that several towers may be used with one calibrated electrode and the platinized surfaces are only in intermittent contact with the alkali. A side arm communicates with the tower via a stopcock. During absorption the side arm is closed by a rubber stopper and the stopcock is shut. A rigid pattern of dipping electrode (E), with platinized platinum plates (B) is immersed in the absorbent which, for the purpose of reading, was transferred to the side arm by appropriate manipulation of the gas stream and stopcocks. Mixing was effected by raising and lowering the liquid as in the type (1). During

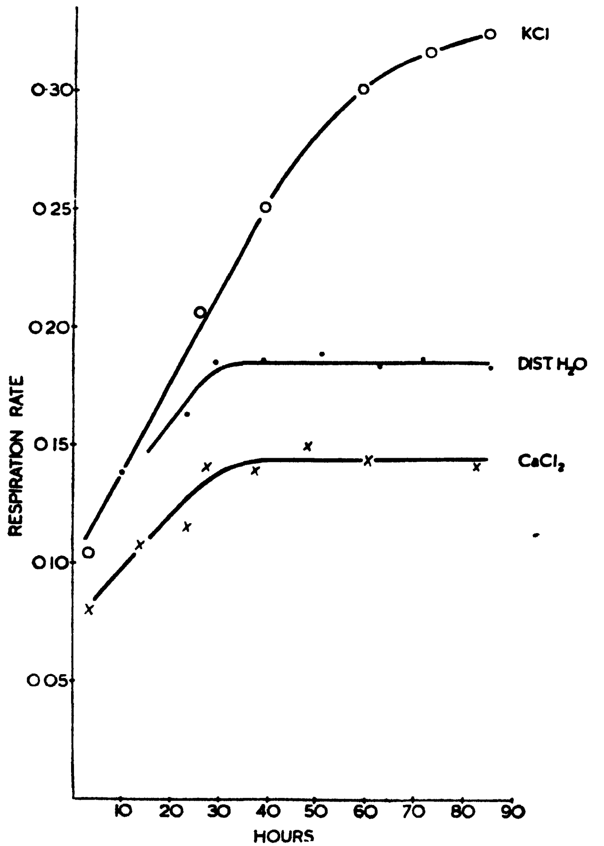


FIG. 3. Effect of salts (0.050 equivalents per liter) and of time on the respiration of potato discs at 23° C.

mixing the apparatus beyond the tower was isolated by a screw clip and the pressure before the tower released, by the slow passage of the accumulated gas to air, through capillary resistance again attached as a side arm by a T-piece.

EFFECT OF SALTS AND OF TIME ON THE RESPIRATION OF POTATO DISCS

Using the above method for carbon dioxide determination the effect of time on respiration was determined for parallel cultures of 50 discs (average thickness approximately 0.75 mm.) placed in distilled water, 0.05 equivalent KCl, and 0.05 equivalent $CaCl_2$ respectively. Figure 3 shows clearly that after about 25 to 30 hours, during which a rising respiration rate obtained as the tissue became adjusted to the conditions of temperature and aeration, a steady level of respiration (0.186 mg. CO_2 per gm. per hour) was attained. Similar tissue in 0.05 equivalents potassium chloride per liter, and at the same oxygen concentration and temperature, finally attained a respiration rate 1.75 times as great as that in distilled water; whereas in calcium chloride of the same equivalent strength, the steady level attained was at 0.144 mg. CO_2 per gm. per hour. The behavior in time of discs in distilled water has long been a familiar feature of this work (**32**, **37**) and, since all the factors affecting respiration were not known, it has been recognized that the plateau in the time curve was due to some factor, other than oxygen concentration, which was in relative minimum (**32**). It is clear from these curves that the essential factors concerned, which regulate the pace of the respiration of the tissue, are conditioned by the salt concentration in the external solution. The opposed action of potassium and calcium salts with a common anion indicates that it is the cations which are the causal agencies. The elucidation of this effect must await description of the other detailed metabolic effects caused by the salt treatment.

CHANGE IN CALORIFIC VALUE

The energy value of the processes of metabolism should be measured when the ability of cells to carry out processes which require energy is in question. Ideally this requires the measurement of both the total energy lost by the metabolizing cells in the form of heat and also of the change in the calorific value of the cells. Assuming that the cells do work the tissue should decrease in calorific value in excess of the energy which is lost merely in the form of heat and, if these measurements proved to be possible, a direct measurement of the energy converted into work by the tissue might be made. At the outset it must be recognized that this ideal is as yet unattainable but the experiments here described represent a first contribution towards this ultimate objective.

It is a well known fact that at a cut potato surface heat is produced in a quantity sufficient to raise the temperature of the superficial cells above that

of tissue which is deep seated and thus less active (27). Assuming that the bulk of the carbon dioxide production of these potato discs arises from the aerobic breakdown of sugar and that the energy value of this reaction appears in the form of heat in accordance with the equation

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674{,}000 \text{ cal.}$$

then at a rate of respiration of 0.20 mg. CO_2 per gm. per hour—a rate which may be long maintained at 23° C.—one might anticipate a continuous heat production of the order of 22 cal. per hour for cultures of 60 discs (45 gm.). Thus stated the measurement of the respiration of potato discs by its heat production seems to be within the scope of the refined heat measurements which have been used in other types of investigations. The measurement of the heat production of nerve by DOWNING, GERARD, and HILL (7) involved the measurement of heat production of the order of 10×10^{-6} cal. per gm. per second. The differential calorimeter of RANDALL and ROSSINI (25), equipped with multiple junction thermocouples and devices which measure a temperature rise as small as $21/5 \times 10^{-6}$ °C. in a calorimeter of heat capacity of the order of 65 cal. per degree, has been applied by FIFE (9) in an intensive investigation of the heat production and respiration of Azotobacter. FIFE was able to show that the heat produced by Azotobacter was exactly equal to the amount to be expected from the carbon dioxide released. In other words, within the limits of error, *all* of the energy released by those respiratory processes which culminate in carbon dioxide output was accounted for as heat and the source of the energy needed by the organism remained obscure. ALGERA (1) has used an automatic calorimeter (accurate to 1.4 per cent. on a heat production of 20 cal. per hour), in which the heat production of the organism (*Aspergillus niger*) is compensated by evaporation caused by forcing dry air into the system with a special pump. The change in the calorific value of the solution and the mycelium was measured as well as the heat and carbon dioxide evolved. The data led to the surprising conclusion that the energy derived from the respiration of glucose to carbon dioxide and water is not involved in the synthetic processes of the growing fungus but it is dissipated in the form of heat.

Direct attempts, however, to measure the continuous heat production of potato discs under the conditions conducive to salt accumulation were abandoned for the following reasons. Of necessity the tissue had to be immersed in a relatively large volume of solution and this increased the heat capacity of the culture vessel to such an extent that the most sensitive type of thermal measurement would be necessary. The correction imposed by the heat leak of the apparatus, a correction which even the best of large wide-mouthed Dewar flasks entail, was further complicated by the additional corrections due to the stirring system (the heating effect due to the mechanical stirrer and the conduction due to necessary metal parts) and also to the effect of the rapid air stream used for the supply of oxygen. It became apparent

that on a maintained heat production of the order of 22 cal. per hour, and with a calorimeter with a heat capacity of the order of 2 to 3 kgm. cal., the expected heat production was too near the order of the various corrections incidental to the technique and the apparatus to make this a profitable line of approach, at least in the early stages of the problem.

Attention was therefore confined to the second part of the heat problem, namely, the measurement of the total heat changes as shown by the calorific value of the discs. If the theoretical amount of heat from the conversion of sugar to carbon dioxide and water is to be regarded as lost by the tissue to the surroundings (and the justification for this now rests mainly upon the work of FIFE and ALGERA) then comparison can be made between this quantity and the observed decrease in calorific value. Thus a first picture could be obtained of the energy exchanges involved in the metabolism of potato discs. Whereas the direct measurements of the continuous heat production of the immersed living discs would tax even the most refined technique, preliminary observations showed that the over-all change in calorific value caused by a period of 88.25 hours of metabolism at 23° C. was readily measurable, as shown by the data of table V obtained from discs treated under the standard conditions of the salt accumulation experiments.

These figures, which could be supplemented by many others of a similar nature, serve to demonstrate at once the very considerable magnitude of the heat changes which occur in the discs and also to present the problem that the loss of heat thus observed was *much in excess of that to be anticipated from the measured carbon dioxide production.* It seemed, therefore, that the determination of calorific value could be profitably applied to the problem of salt absorption and metabolism.

The measurements of calorific value in table V, were made using an

TABLE V

CHANGE IN CALORIFIC VALUE DURING METABOLISM OF POTATO DISCS*

SAMPLE	CONDITIONS	FRESH WEIGHT 40 DISCS	VACUUM DRY WEIGHT 40 DISCS	CALORIES PER GRAM DRY WEIGHT	TOTAL CALORIES	CHANGES DURING METABOLISM: CALORIES OBSERVED	RESPIRATION: CO_2	RESPIRATION: CALORIES CALCULATED
		gm.	*gm.*	*cal.*	*cal.*	*cal.*	*mg.*	*cal.*
Initial	....	32.50	5.296	4054	21,480	....	....	....
Final	Distilled water	34.90	4.456	4207	18,750	2730	687.2	1755
Final	0.001 N KBr	35.95	4.539	4176	18,910	2570	730.2	1865

* 40 discs exposed to 2 liters of solution under standard conditions (33) at 23° C. for 88.25 hours. Solutions aerated by washed air at 15 liters per hour.

EMERSON calorimeter (a standard form of gold lined bomb calorimeter) with an oxygen pressure of 20 atmospheres. Thermocouple methods for the determination of the heating and cooling curves of the bath, before and after combustion, were not employed but the BECKMANN thermometer which was used was especially calibrated over the necessary part of the scale. An electrical vibrator activated at the time of each reading, prevented the meniscus from adhering to the walls. The determinations were made on amounts of approximately 1.0 gm. of dried, ground, and uniformly sieved potato tissue. Special attention had to be given in all of this work to the determination of dry weight and the redrying which must precede the removal of aliquot parts of dry matter for all analyses. For calorimetrical work the potato tissue was not pressed into a pellet but was mixed with sufficient magnesium oxide and ignited electrically on a small boat—thus the formation of small incompletely burnt pellets of carbon was prevented. The heat capacity of the calorimeter and bomb (2200 cal.) determined from the combustion of 1.0 gm. of sugar or benzoic acid, included a standard volume of water within the bomb which was sufficient to dissolve soluble products of combustion. The temperature of the water bath which contained the bomb was maintained uniform by a stirrer, the speed and operation of which were rigorously controlled. Special care was taken to procure symmetrical heating and cooling curves. The order of the expected temperature rise could be estimated in the light of experience and the weight of water in the bath and its initial temperature were accurately adjusted so that prior to ignition a smooth heating curve was obtained and finally a smooth cooling curve from a temperature approximately as much above room temperature as the bath temperature at the time of firing was below. These conditions permit the best results from the application of certain empirical constants in the calculation.

Prior to firing, the heating curve of the water bath was obtained by readings every half minute; the time of firing was noted and the course of the ensuing temperature rise and subsequent fall was similarly recorded. The observed maximum temperature increase was corrected for the thermometer error and also by a "radiation correction" calculated from the empirical formula suggested by SCOTT (29) in his description of the determination of the calorific value of coal. The calculated correction compensates for the errors due to radiation and the time lag in the response of the water bath. Unburned fuse wire, if any, was weighed and the appropriate correction applied. Under the best conditions combustions which involved the production of approximately 4000 cal. could be repeated on successive samples to within [illegible] cal.[4] In all cases where values are assigned to tissue samples in this and following papers the figure given is the mean of concordant, replicate determinations and its accuracy is of the order named.

[4] This accuracy was due principally to the care and time expended on these determinations by P. R. STOUT of the division of Plant Nutrition, University of California.

Carbohydrate and nitrogen metabolism

It was essential that the scheme of analysis should reveal the hydrolysis of starch to sugar and its utilization in the metabolism of the discs. Though ultimately desirable, a complete analysis of the various hexose sugars has not yet been attempted; the carbohydrate analyses were confined to the determination of starch and total sugar. Furthermore, to economize material and time it was necessary to make as many determinations as possible upon a single extract. The essential nitrogen and carbohydrate data were therefore obtained by the following scheme:

(1) Potato discs were killed, either by heating for 1 hour at 100° C. followed by drying to constant weight at 60° C. *in vacuo*, or by boiling on a water bath for 20 minutes in excess of alcohol at a final concentration of 70 per cent. by volume.

(2) Complete extraction of either alcohol killed discs or vacuum dry tissues by 70 per cent. alcohol was carried out on an electric water bath with reflux condenser.[5] The accumulated alcohol extracts were evaporated to dryness, the residue dissolved in water and made up to standard volume. On aliquot parts of this solution the total nitrogen (alcohol soluble N) was determined by micro-Kjeldahl methods (**24**) and, after clearing[6] with neutral lead acetate, deleading with oxalic acid (**17**), and acid (3 per cent. HCl) inversion, the total sugar was determined by the Munson-Walker gravimetric method (**6**).

(3) The residue from alcohol extraction was dried, weighed, and used for the determination of alcohol insoluble nitrogen (protein N) and starch. The starch was digested with a salivary extract under toluene, alcohol extracted, the soluble products of hydrolysis inverted, the sugar determined by the Munson-Walker method, and the result calculated in terms of glucose (**2, 3, 23, 42**).

A full record of the fate of the carbohydrates during the metabolism of potato discs will be given in a subsequent paper. It is necessary only to remark here that discs, cut from dormant tubers and washed in running tap water for 24 hours, commonly have a total sugar content of about 3.0 mg. per gm. fresh weight (table III) whereas after 72 hours in aerated distilled water the concentration of sugar increases to 8.0 mg. per gm. This confirms the evidence of starch hydrolysis which can be obtained microscopically.

The scheme needs further comment with reference to the nitrogen determinations. The alcohol-soluble nitrogen will be designated "soluble N"

[5] For special purposes (see under amide-nitrogen) a "cold alcohol extract" is desirable. This was made at about 5° C. in a refrigerator.

[6] In work in which the changes in sugar concentration, rather than absolute values, are involved the clearing technique can be omitted, as the amount of non-sugar material in potato determined by the Munson-Walker method is small (order of 2 per cent. in "final" tissue).

and the alcohol-insoluble nitrogen will be identified as "protein N." This is subject to the reservation that the potato is relatively free from alcohol-soluble proteins and alcohol-insoluble non-protein nitrogen. It was anticipated that during the metabolism of the discs protein synthesis would occur and, therefore, that the alcohol-insoluble fraction would increase at the expense of the alcohol-soluble portion. A test of the recovery of the total nitrogen would be the constant sum of the two fractions. Difficulty was never encountered with the accurate recovery of total nitrogen, mainly because the discs do not lose nitrogen appreciably to aerated solutions.

Since many of the experiments to be described did show protein synthesis by the alcohol method the following evidence is quoted because it confirms that the *changes* in the alcohol-insoluble nitrogen really did measure the change in protein content. In table VI are given the changes in nitrogen

TABLE VI

PROTEIN SYNTHESIS BY POTATO DISCS. COMPARISON OF THE ALCOHOL AND TRICHLORACETIC ACID METHODS

SERIES	SALT SOLUTION	SAMPLE	BY CCl_3COOH METHOD		BY ALCOHOL METHOD	
			PROTEIN N PER GRAM INITIAL FRESH WEIGHT	CHANGE IN PROTEIN N CONTENT	PROTEIN N PER GRAM INITIAL FRESH WEIGHT	CHANGE IN PROTEIN N CONTENT
			mg.	*mg.*	*mg.*	*mg.*
A	....	Initial	0.66		0.77	
	0.075 equiv. KBr			+ 0.65		+ 0.65
		Final	1.31		1.42	
B	....					
	0.075 equiv. $CaBr_2$	Initial	0.66		0.74	
				− 0.14		− 0.09
		Final	0.52		0.64	

content of potato tissue which in series A had been exposed for 63.1 hours to aerated KBr solution and in series B had been exposed for 87.3 hours to aerated $CaBr_2$ solution at 23° C. At this stage these treatments need concern us only in that one produced a significant gain of protein and the other a slight loss. The samples were analysed both by the alcohol method and also by the use of trichloracetic acid as a protein precipitant and thus, by comparing the initial and final discs, independent estimates of the change of protein content could be calculated.

It will be seen from the table that the fact of protein synthesis in potato discs exposed to aerated solutions of potassium salts is established. This is not the place to enlarge upon the contrast between the results in the different salt solutions except to note that the effects of the salt on synthesis of protein are parallel to those already described on respiration.

Clearly the different methods yielded almost identical results. The discrepancy, though small, is real and the trichloracetic method yielded protein values which were lower than those by the alcohol method. The estimates of *protein synthesised* were unaffected, however, by the method of analysis (series A). It should be mentioned, however, that further difficulty has been encountered in the analysis of tissue rich in phosphate after absorption in strong phosphate solutions. In this case the alcohol method is unaccountably at fault and yields values which are too low; such difficulties may be overcome by the use of the trichloracetic acid procedure. The method adopted, then, is as follows. The dried, ground tissue is redried at 60° C. *in vacuo*. Aliquot parts of about 50.0 mg. are transferred to 50 ml. pyrex beakers and stirred with 20 ml. of 2.5 per cent. trichloracetic acid for about 15 minutes. The insoluble residue containing protein is then filtered, washed, and transferred on the paper to a Kjeldahl flask in which it is digested in the usual way.

Nature of the compounds concerned in protein synthesis

The reality of protein synthesis in thin aerated potato discs under the standard conditions conducive to salt accumulation can be sufficiently established by reference to the fractions designated "soluble nitrogen" and "protein nitrogen" (table VI, series A). Later papers will be greatly concerned with the effect of different variables upon the process of synthesis. It is, therefore, necessary to specify in more detail the compounds which are involved. The soluble nitrogen fraction is a composite one and includes both amino acids and amides. Free ammonia is a negligible component of the soluble nitrogen except when the tissue has absorbed large amounts of nitrate, or has been so treated during analysis that ammonia arises from the breakdown of relatively unstable soluble nitrogen compounds (amides) which are normal constituents of the potato tissue.

The first point which arises, and one which assumes greater importance in the light of the relationship between respiration and nitrogen metabolism to be discussed later, is the relative contribution of amino acids and amides to the nitrogen used in protein synthesis.

This question may be answered directly by reference to the results of the further fractionation of the soluble nitrogen fraction and, indirectly, by reference to the changes caused in protein synthesis in the titration curve of the sap extracted from the frozen tissue. Before dealing with the type of nitrogen compound concerned in protein synthesis, however, the existence in the actively metabolizing potato discs of unstable nitrogen compounds and their effect upon the fractionation of the "soluble nitrogen" must be appreciated.

Unstable nitrogen compounds.—The soluble nitrogen fraction obtained by alcoholic extraction of the fresh discs contained nitrogen compounds of

very different degrees of stability. It was observed that the evaporated alcoholic extracts contained unexpectedly large amounts of ammonia which can be attributed to "easily hydrolyzable amides" of the kind which VICKERY (43) has shown may be hydrolyzed at pH 7.0 by boiling for two hours. The following evidence establishes that unstable amides are real components of potato tissue and also shows their relation to protein synthesis.

Extracts of the soluble nitrogen of potato tissue were made in two ways: a "hot extract" made from the fresh tissue by boiling in 70 per cent. alcohol (pH 5.8 to 6.0), and a "cold 70 per cent. alcoholic extract" made at 5° C. from the dried tissue. The amino-nitrogen and ammonia-nitrogen content of these extracts calculated to the initial fresh weight of the discs (table VII) clearly show that the method of hot extraction released ammonia from some component of the soluble nitrogen fraction which, though stable in cold 70 per cent. alcohol, decomposed on boiling for several hours.

Table VII shows that the unstable substance which released ammonia in the hot extracts was amide since the amide content of the cold extracts (by hydrolysis with 6N HCl for 3 hours suitably corrected for the free ammonia obtained by distillation with magnesia) exceeded that of the hot extracts by an amount equivalent to the extra ammonia the latter contained. VAN SLYKE determinations made on the hot and cold extracts showed, however, that there was also an apparent loss of amino-nitrogen in the hot extracts. The "apparent" amino-nitrogen which decomposed as a result of prolonged, hot (100° C.) extraction at the pH of the tissue extract (5.8 to 6.0) corresponded to 80 to 90 per cent. of the unstable nitrogen present and *did not reappear as ammonia,* since the latter could be ascribed entirely to easily hydrolyzable amide. These results can be explained in the light of the work of VICKERY, CHIBNALL *et al.* (44) on the hydrolysis of glutamine. It has been shown that the amide group of glutamine yields ammonia quantitatively (over 98 per cent.) after 2 hours hydrolysis at pH 6 to 7) and moreover that about 80 to 90 per cent. of this amide group also contributes to the "apparent amino nitrogen" which is determined by the VAN SLYKE method. Further, during the hydrolysis of the amide glutamine, there is a concomitant loss of the amino group without the formation of ammonia so that for glutamine the decrease in "apparent amino nitrogen" tended to be double the ammonia released from the amide group. The combined effect of these processes is that the quantitative liberation of ammonia from the amide group of glutamine, which occurs in weakly acid or neutral solutions in two hours, is accompanied by a decrease in the "apparent amino nitrogen" content. VICKERY, CHIBNALL, *et al.* (44) emphasize that although a simultaneous loss of "apparent amino nitrogen" accompanying the quantitative hydrolysis of the easily hydrolyzable amide is strong quantitative evidence that the amide concerned is glutamine (or same other substance ca-

TABLE VII

EFFECT OF SALTS AND AERATION ON THE NITROGEN FRACTIONS OF POTATO DISCS. ALL QUANTITIES OF NITROGEN IN MG. PER GRAM OF INITIAL FRESH TISSUE

EXPERIMENTAL TREATMENT OF DISCS	PROTEIN N	SOL. N	AMINO N			AMIDE N			AMMONIA		
			HOT EXT.	COLD EXT.	APPARENT "UNSTABLE"	HOT EXT.	COLD EXT.	EASILY HYDROLYZABLE	HOT EXT.	COLD EXT.	$NH_4 \equiv$ TO UNSTABLE AMIDE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	0.92	1.15	0.90	1.04	0.14	0.129	0.305	0.176	0.178	0.002	0.176
53.0 hours in aerated 0.075 M KCl at 23° C.	1.21	0.85	0.57	0.675	0.105	0.156	0.270	0.114	0.120	0.002	0.118
Original washed discs	0.68	1.38	1.09			0.208	0.348	0.140	0.144	0.002	0.142

pable of forming a pyrrolidone ring) the value of the ratio between these two quantities is too variable to be of use for quantitative measurement. The ratio of the decrease in apparent amino nitrogen to the decrease in easily hydrolyzable amide was observed by VICKERY, CHIBNALL, *et al.* (**44**) to vary from 0.5 to 1.24 for different derivatives of glutamic acid.

Table VIII shows that the "easily hydrolyzable amide" which was de-

TABLE VIII

CONTENT OF UNSTABLE NITROGEN COMPOUNDS IN POTATO DISCS. ALL QUANTITIFS OF NITROGEN IN MG. PER GRAM OF INITIAL FRESH WEIGHT

EXPERIMENTAL TREATMENT OF DISCS	APPARENT UNSTABLE AMINO-N	EASILY HYDROLYZABLE AMIDE	NH_3 FROM UNSTABLE N COMPOUNDS	APPARENT UNSTABLE AMINO N / EASILY HYDROLYZABLE AMIDE × 100
	mg.	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	0.14	0.176	0.176	79.5
53.0 hours in aerated 0.075 M KCl at 23° C.	0.105	0.114	0.118	92.0

composed during alcoholic extraction yielded ammonia quantitatively but that the parallel loss of VAN SLYKE amino nitrogen corresponded to only 80 to 90 per cent. of this and therefore only to the amount reasonably expected from an unstable amide group, such as that contained in glutamine, which also reacts in the VAN SLYKE method. With this reservation and in the light of the statement of CHIBNALL and VICKERY referred to, these data establish a strong presumption that the unstable amide is either glutamine, or a closely related compound. The isolation and complete identification of this unstable amide present in actively metabolizing potato discs will be a necessary part of future investigations. If the "unstable amide" were contained in glutamine, the true amino nitrogen of the hot extracts would be less than that of the cold extracts and the nitrogen concerned in this change would not be estimated in any of the fractions determined. The sum of the amino-nitrogen (of the hot extracts), however, the stable, and the unstable amide, do account for the total soluble nitrogen. It appears, therefore, that the amino groups, if any, of this glutamine-like amide are too stable for this substance to be glutamine itself.

DETERMINATION OF AMIDE AND AMINO NITROGEN IN POTATO DISCS.—Clearly,

therefore, the true measure of the *total amide* content of potato discs is obtained only by the determination of this group by acid hydrolysis at pH 0 for 3 hours on the cold extract of tissue dried *in vacuo* at 60° C. This hydrolysis was appropriately corrected for the free ammonia obtainable by distillation with magnesia. The difference between the figure for total amide and that obtained by hydrolysis at pH 6 for 2 hours (which equals the gain in ammonia content in the hot extracts) is a measure of the easily hydrolyzable amide. The free ammonia content of the discs is obtained from either the cold extract or by direct distillation of the dry powder with magnesia. A measure of the amino nitrogen[7] (free from interference due to the unstable amides) is obtained by the application of the VAN SLYKE method to the hot alcoholic extract. Table IX shows that these fractions

TABLE IX

SOLUBLE NITROGEN OF POTATO DISCS. ALL QUANTITIES IN MG. PER GRAM OF INITIAL FRESH WEIGHT

EXPERIMENTAL TREATMENT OF DISCS	TOTAL SOL. N	AMINO N	TOTAL AMIDE	NH_4	AMINO + AMIDE + NH_4OH NITROGEN
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	1.15	0.90	0.305	0.002	1.207
53.0 hours in aerated 0.075 M KCl at 23° C.	0.85	0.57	0.270	0.002	0.842
Original washed discs	1.38	1.09	0.350	0.002	1.442

account accurately for the total soluble nitrogen of the tissue. Since the amide fraction, which is stable in neutral or weakly acid extracts, is probably asparagine a probable figure may be derived for the "non-asparagine amino nitrogen" by deducting from the total amino nitrogen (determined as above) an amount equivalent to the stable amide fraction. This is probably the best measure available from these data of the amino content of the free amino acids.

SOURCE OF THE NITROGEN INVOLVED IN PROTEIN SYNTHESIS.—Table X compares the changes in the soluble nitrogen and its component fractions with the gain of protein nitrogen per gram of initial fresh weight which occurred in potato discs during experiments in which they were exposed to dilute salt solutions and the standard conditions conducive to salt accumulation and

[7] Excluding the amino group of glutamine, if present, but including that of asparagine.

TABLE X

NITROGEN FRACTIONS INVOLVED IN PROTEIN SYNTHESIS IN POTATO DISCS. ALL AMOUNTS OF NITROGEN IN MG. EQUIVALENTS PER GRAM INITIAL FRESH WEIGHT

Experimental solution		Hours	Gain protein N	Loss soluble N	Loss of NH_2-N		Loss of non-asparagine amino nitrogen		Loss of total amide		Loss of stable amide (asparagine?)		Gain of unstable amide		Loss of NH_2-N + total amide	
Salt	Concentration equivalents per liter				Amt.	As per cent. of loss of sol. N	Amt.	As per cent. of loss of sol. N	Amt.	As per cent. of loss of sol. N	Amt.	As per cent. of loss of sol. N	Amt.	As per cent. of loss of sol. N	Amt.	As per cent. of loss of sol. N
			mg.	mg.	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
$CaCl_2$	0.00075	53.1	0.18	0.18	0.14	78	0.10	55.6	0.008	4.5	0.041	22.8	0.033	18.3	0.15	83
KCl	0.00075	52.5	0.24	0.23	0.19	83	0.12	52.3	0.029	12.6	0.074	32.1	0.045	19.5	0.22	96
KCl	0.075	53.0	0.55	0.53	0.52	98	0.47	90.6	0.078	14.7	0.052	9.8	-0.03	-5.6	0.60	112
$CaBr_2$	0.00075	88.0	0.35	0.39	0.36	92	0.21	53.9	0.082	21.0	0.15	38.5	0.068	17.4	0.44	113
KBr	0.00075	63.5	0.28	0.29	0.31	107	0.21	72.5	0.021	7.3	0.10	34.5	0.079	27.2	0.33	114
KBr	0.075	63.0	0.65	0.61	0.56	92	0.45	73.7	0.118	19.4	0.11	18.0	-0.08	13.1	0.68	111

high respiration. The agreement between the gain of protein nitrogen and the decrease of soluble nitrogen is close. The table shows (column 6) that almost the entire amount of the soluble nitrogen used in protein synthesis was derived from the amino nitrogen fraction and when the maximum[8] allowance is made for that part of the amino nitrogen which might have been derived from the asparagine metabolized (column 7) it is clear that amino compounds other than asparagine, which in the light of the titration curve data must be amino acids, contribute most of the nitrogen used in the synthesis of protein by the potato cells. The table also shows certain specific effects of the salts. The stronger potassium chloride solution increased the protein synthesis. This confirms a result which has already been noted (table VII). The table, however, shows that the effect of the salt (KCl) on protein synthesis could have been entirely at the expense of the reserve of non-asparagine amino (amino acid) nitrogen. In the presence of dilute calcium solutions the non-asparagine amino nitrogen contributed much less of the total used in protein synthesis than when the tissue was in contact with the stronger aerated potassium salts (column 7).

The conclusion, therefore, is that the nitrogen involved in protein synthesis is drawn principally from amino compounds (amino acids) other than asparagine or glutamine but also that the salts present affect not only the total amount of protein synthesized but also the source from which the nitrogen is obtained. Increased concentrations of potassium salts, which increase the total synthesis, also increase the relative utilization of the amino acids whereas the calcium salts, which depress the total synthesis, also decrease the utilization of the amino acids relative to other compounds.

The status of the amide fraction of the soluble nitrogen is complicated by the formation during the period of active metabolism of easily hydrolyzable amides. Protein synthesis (table X) was also accompanied by a decrease in the total amide fraction and this, together with the observed loss of amino nitrogen, accounts for the entire change in soluble nitrogen with an error which is probably not excessive in view of the complications in these determinations. The change in the total amide, however, conceals a much greater utilization of the more stable amides (asparagine) which is offset by the accumulation, in the actively metabolizing tissue, of easily hydrolyzable amides. In the light of the evident connection between respiration and nitrogen metabolism, the extreme reactivity of this substance, its apparent increase when the conditions of oxygen and temperature permit rapid metabolism (*e.g.* in dilute salt solutions), and its further depletion when, in strong potassium salts, protein synthesis is stimulated, are all features which might suggest that this substance may be a reactive intermediary between the stable reserves of amino acid amide on the one hand and protein on the

[8] If any of the disappearance of "stable amides" is accounted for by the conversion of asparagine to aspartic acid, which remains as such, this correction is too great.

other. These considerations arouse an interest in the nature and reactions of this glutamine-like component of the soluble nitrogen fraction which is not overshadowed by the greater abundance in the tissue of the more stable nitrogenous substances.

Titration curves of potato sap

The determination of titration curves on the expressed sap of plants reveals the effect of experimental treatments on the constituents of the buffer complex. Since the components of the buffer system include certain substances which are not readily determined quantitatively (*e.g.* amino acids and organic acids) this determination has considerable value. For this purpose, however, the buffering in the region of the pH of the sap—with which most investigations have been concerned—is not especially instructive and the titration must be extended far beyond this to reactions both more alkaline and more acid. See Small (**32**, pp. 265–291) for a discussion of the buffering of potato sap between the limits pH 4.5 to 7.0. Dunne (8) has utilized this method of approach in connection with problems of mineral nutrition, and Hoagland and his collaborators have freely utilized it as an indirect means of investigating the chemical changes which accompany the uptake of salt in barley roots. The authors were familiar with the latter investigations and hence applied this technique in the case of potato discs.

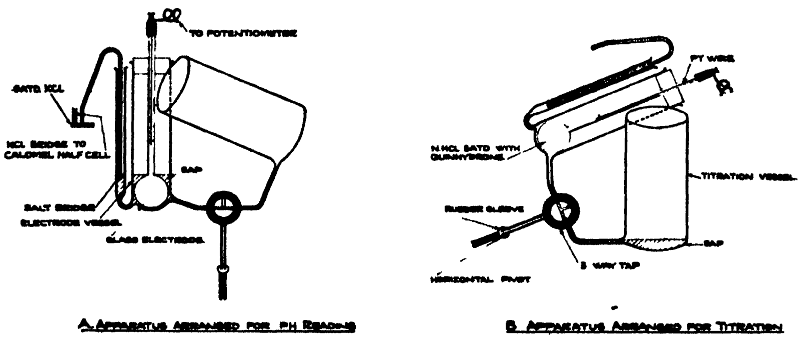


Fig. 4. Rocking electrode and titration vessel for the determination of titration curves of sap.

The titration curves were carried out on aliquots of potato sap freshly expressed from discs which had been frozen and stored at $-20°$ C. after the conclusion of the experimental treatment they received. The change of pH with the addition of 0.20 N HCl, between the pH of the sap and about pH 2.5, was recorded; for the alkaline range to pH 11.5 the treatment was repeated on another aliquot of the sap using 0.20 N NaOH. The amount of acid or alkali added was arbitrarily adjusted according to the shift in pH obtained in the region concerned. The earlier curves (figs. 6 and 7) were

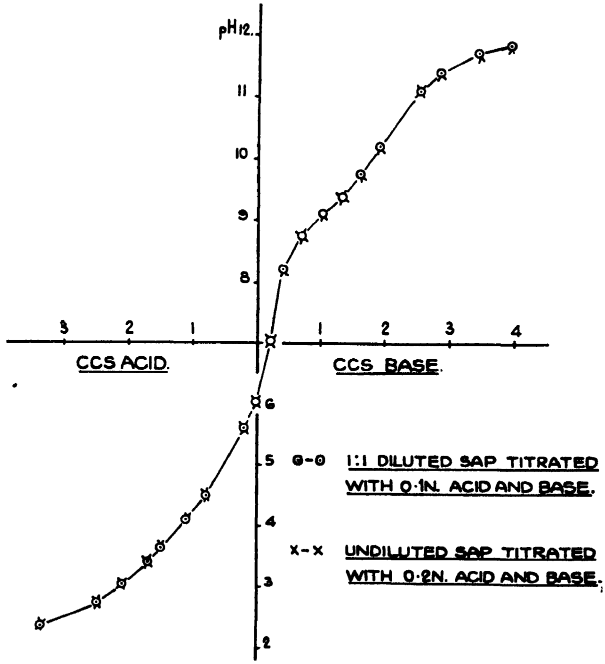


FIG. 5. Titration curve of potato sap.

made from readings obtained using a hydrogen electrode by A. ULRICH[9] although subsequent determinations made with a glass electrode show that the type of curve obtained is not dependent upon a particular method of recording the E.M.F

The titration curve of potato sap can be more rapidly and effectively explored by the use of a glass electrode system in conjunction with a potentiometer of the Electrometer Valve pattern of the Cambridge (England) Instrument Co. This instrument permits readings to be obtained which are automatically corrected for temperature and which can be stated in E.M.F. or pH units with an accuracy which corresponds to 0.01 pH unit. A convenient form of combined electrode and titration vessel is that described in figure 4 A and B. The necessary additions of acid or alkali are made (from burettes reading to 0.01 ml.) to the sap sample which is contained in the titration vessel, placed for the purpose in the position of figure 4 A. In the position shown in figure 4 B the liquid undergoing titration flows by gravity into the glass electrode chamber and covers the bulb of the electrode. The combined electrode and titration vessel rocks from the position of figure 4A to that of figure 4B on an axis provided by a horizontal portion of the outlet tube. Repeated passage of the liquid from the electrode chamber to the

[9] Division of Plant Nutrition, Berkeley, Cal.

titration vessel insures efficient mixing. The glass electrode bulb contains N HCl, saturated with quinhydrone into which dips a platinum wire. The electrical connection between the glass electrode vessel and the calomel half cell is made (in the position of fig. 4B) by a salt bridge and a saturated potassium chloride solution.

The combined rocking electrode and titration vessel mounted on a hard wood base was so designed that it worked with a minimum of 4 ml. of liquid. To economize material, 2 ml. of sap plus 2 ml. of water were used for each of the titrations with acid and base. It was verified experimentally that dilu-

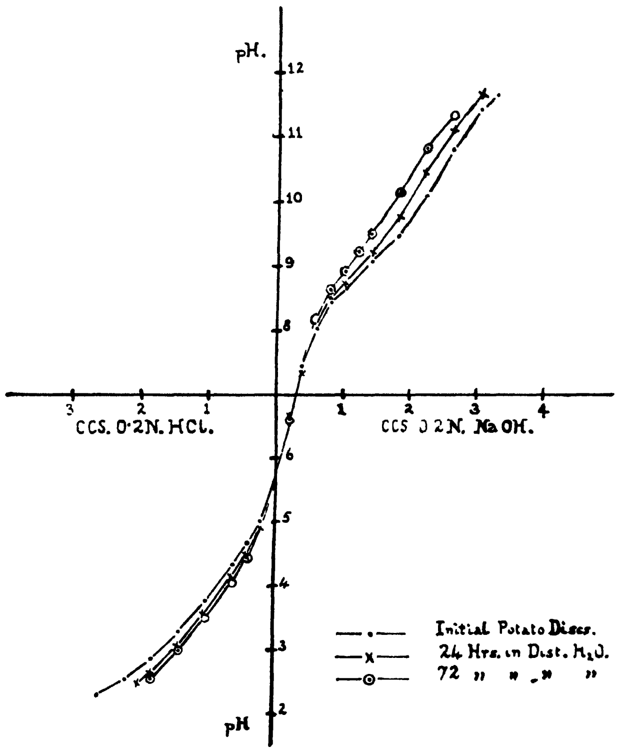


FIG. 6. Changes in the buffer system of potato discs during metabolism at 23° C. in aerated distilled water—the effect of time.

tion in the ratio of 1:1 did not change the nature of the buffer system of the sap. This is evident from figure 5 which shows the titration curves of undiluted potato sap using 0.2 N acid base and also of sap diluted 1:1 with 0.1 N acid and base.

EFFECT OF SALTS AND METABOLISM ON THE BUFFER SYSTEM OF POTATO

The effect on the titration curve of potato sap of the metabolic processes which occur in the discs in distilled water, and under the conditions of

temperature and aeration conducive to maximum respiration and salt accumulation was determined. In figure 6 can be seen the titration curves of the sap which was expressed after freezing from the initial discs, prior to their period of intense metabolism, and also after periods of 24 and 72 hours, respectively, of high respiration.

Normally the sap of the potato is most strongly buffered between pH 8.5 to 9.5 (figs. 5 and 6), the region in which the buffer effect of amino acids is at its maximum.[10] Progressively with time the metabolic processes in the

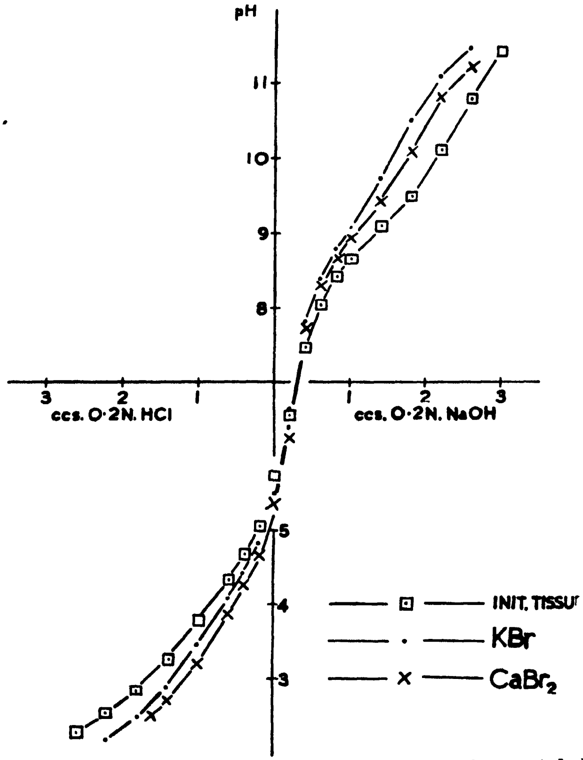


FIG. 7. Changes in the buffer system of potato discs during metabolism at 23° C. in aerated solutions—the effect of salts (KBr, $CaBr_2$).

thin, aerated discs in water cause a decrease in these buffer components which is to be correlated with their conversion to substances with little buffer value, *e.g.*, protein. As figure 7 shows the decrease of constituents which buffer between pH 8 and 10 is greater when the tissue is in contact at 23° C. with aerated solutions of potassium salts than calcium salts—a result which is in agreement with the finding that the former accentuate and the latter

[10] The pKa values for some typical amino acids are as follows: glycine 9.7, aspartic acid 9.9, glutamic acid 9.8, tyrosine 9.4, phenylalanine 8.6.

depress protein synthesis from the stored amino compounds of the potato tissue.

Since the changes which occur in that portion of the titration curve which lies between the limits pH 8.0 to 10 are closely related to the metabolism of the cells, it is desirable to specify the substances involved. Further proof that amino acids are responsible is provided by the data in figure 8,

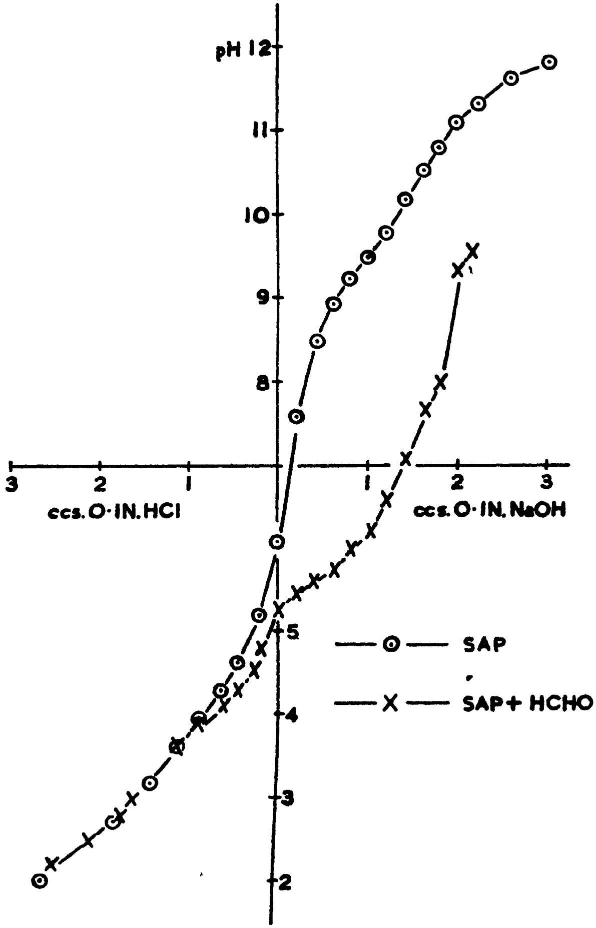


FIG. 8. Effect of formaldehyde on the titration curve of potato sap.

which shows the titration curve of diluted potato sap (2 ml. sap plus 2 ml. H_2O) with and without the addition of neutralized formaldehyde (4 ml. of 44 per cent.). In the acid reactions (more acid than pH 4.0) the two curves coincide, but in presence of formaldehyde the buffer capacity normally found

in the region pH 8 to 10 is displaced to the region pH 5.0 to 6.5—a region in which the sap is normally only weakly buffered. The compounds involved must therefore be amino acids which in presence of formaldehyde form methylene-imino derivatives which, being stronger acids, have their maximum buffer effect at a lower pH (pKa values of the methylene-imino derivatives of common amino acids range from 5.4 to 6.9).

It still remains to determine whether the amino-acid moiety (pKa = 8.87) of the asparagine present in potato tissue could account for the changes which occur in the buffer value in the region of pH 8 to 10 during metabolism. SMALL (30) estimated that asparagine contributed only 5 per cent. of the buffer value of potato sap in the region pH 6.7. Figure 9, which

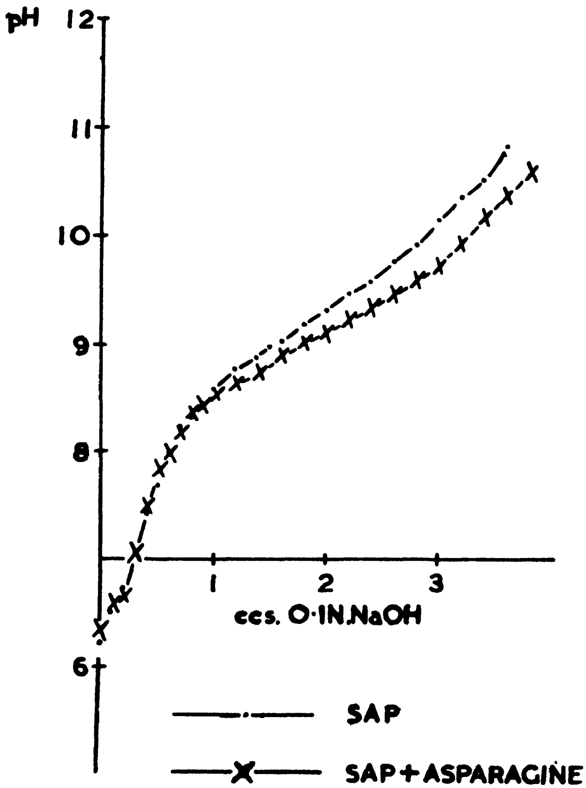


FIG. 9. Effect of added asparagine on the titration curve of potato sap.

shows the titration curve of 2 ml. of potato sap, with and without the addition of an amount of asparagine such that its amino-N content was equal to the total soluble nitrogen of the normal sap. It is evident that *per unit of soluble nitrogen* the normal sap has much greater buffer value than the amino-acid moiety of the asparagine which it contains.

The conclusion, therefore, is that the substances which buffer the normal sap strongly in the region of pH 8 to 10 are amino acids other than asparagine and that the changes induced in this part of the titration curve due to the combined effects of alkali halides and metabolism reflect the effect of these treatments upon the amino acid content of the tissues. This conclusion is in agreement with that obtained by the direct determination of the components of the soluble nitrogen.

At reactions more acid than the natural sap there is a considerable degree of buffering which must be attributed to organic acids. According to SMALL (30) citrates and phosphates might contribute 60 per cent. of the buffering between pH 6 and 7 where, however, the buffering is weak; but at reactions more acid, their contribution is much less *e.g.*, 19 per cent. at pH 4.5. SMALL was led to the conclusion that even at reactions as near neutral as pH 5.0 other organic acids were involved and he demonstrated the presence of ether soluble organic acids. From the curves given in figures 5, 6, and 7 it seems clear that the strongly buffered sap at a pH more acid than 5.0 must owe this property to organic acids and their identification is an imperative task for the future.

The progressive metabolism which occurs in aerated distilled water is accompanied by a continual decrease in the buffer value of the sap at reactions between pH 2.0 and 6.0, and this must mean that organic acids are being consumed. An effect of salts on the organic metabolism of the potato discs is suggested by the greater decrease of the buffer value between pH 2 and 6.0, which occurs when the tissue is exposed to aerated calcium bromide, than when it is respiring in aerated potassium bromide. This is an example of a phenomenon observed frequently by HOAGLAND[11] and associates on barley roots. Both potato and barley results are consistent with the view that the bromide ion is absorbed from calcium bromide solutions mainly unaccompanied by the cation so that organic anions disappear from the sap and bicar-

TABLE XI

EFFECT OF DRYING ON ASCORBIC ACID CONTENT OF POTATO DISCS

SERIES	FRESH WEIGHT OF SAMPLE	STATE OF TISSUE WHEN EXTRACTED	ASCORBIC ACID PER GRAM FRESH WEIGHT
	gm.		*mg.*
I	7.50	Fresh + NaCN	0.164
	7.80	Dry	0.188
II	7.50	Fresh	0.170
	7.50	Dry	0.172

[11] Results awaiting publication by ULRICH and by HOAGLAND and BROYER privately communicated to the authors.

bonate ions accumulate in the external solution (**36**) which also becomes more alkaline. The titration curves, therefore, not only extend and confirm the conclusions on the nature of the nitrogen metabolism which were obtained from direct determinations but they also suggest that the potato cells metabolize organic acids in amounts which are determined by time and the nature of the salt present. With reference to the rôle of organic acids, the conclusions from the titration curves stand alone, as direct analyses of organic acids have not been made. Clearly the above account does not distinguish between the buffer effect attributable to nitrogen-free organic acids and to organic acid radicles contained in polybasic amino acids.

Behavior of potato oxidase, ascorbic acid, and glutathione in the actively metabolizing discs

Under the conditions conducive to salt accumulation potato discs turn slightly brown owing to the activity of the familiar oxidase system of the potato cells which acts upon phenolic compounds (including tyrosine) stored therein. This behavior, noted earlier (**34, 36, 39**) is sensitive to the oxygen pressures which also affect respiration. It is, therefore, a result of no little interest that the effects induced by salts upon respiration are paralleled by visible effects on the oxidation of phenolic compounds—potassium salts increase and calcium salts decrease the browning reaction. The specific effects of the salt are also increased with concentration. It is clear that the cellular oxidation of phenols and respiratory substrates is similarly effected by salts and oxygen even if the same catalytic mechanism is not involved.

The effect of neutral salts, other than those which contribute to buffer solutions, on the activity of the aerobic oxidase preparations isolated by RAPER and his colleagues from potato tissue does not appear to have been studied in detail. SAMISCH (**28**) made an intensive investigation of the effect of salts on the oxidase which causes darkening of fruits (apricots). However, the experiments of SAMISCH on the extracted enzyme reveal no sign of a contrasted effect of potassium and calcium salts such as the organized living cells of potato reveal. In his work, on the contrary, increased concentrations of alkali (*e.g.*, Na) halides *decreased* the activity of oxidase extracts in presence of catechol.

SZENT GYORGYI (**39, 40**) showed that the substance now known as ascorbic acid could reduce the oxidized products produced by potato oxidase from those substances which contain the catechol group, and believed that ascorbic acid could provide for the reversibility of this catalytic system. The first products (quinones) of the oxidation of the phenolic substances of potato are reduced to the phenolic condition by ascorbic acid, which is in turn oxidized. JOHNSON and ZILVA (**13**) found that the enzyme (phenolase) does not act upon ascorbic acid directly but that this substance is oxidized by those inter-

mediates (orthoquinones) which also deaminate amino acids. From the work of KEILIN and MANN (14) on purified polyphenol oxidase, which is apparently a copper-protein compound, it is also clear that the oxidase oxidizes ascorbic acid only in the presence of catechol.

Accordingly attention was turned to the reducing substances to be found in cells and particularly to two substances (ascorbic acid and glutathione) both of which are commonly supposed to bear a close relation to metabolic activity, to be capable of reversible oxidation and reduction, and which have been freely incorporated into the proposed catalytic systems of cells. The expectation was that increased amounts of the brown, oxidized products might be attributed to, or accompanied by, a decreased concentration of the reduced form of the substances in question. It can be stated at once that no clear relationship of this kind between the browning effects and the behavior of either ascorbic acid or glutathione in the living cells has yet emerged. The observations made, however, present yet another feature of the metabolism of potato discs which is of particular interest since the substances involved are supposed to be connected with, if they are not causal agents for, vital activity.

THE DETERMINATION OF ASCORBIC ACID IN POTATO DISCS

The familiar 2:6 dichlorophenolindophenol titration was applied. The dye was used in solutions of 0.10 gm. in 200 ml. of solution buffered at pH 6.8 with a phosphate buffer. The TILLMANN reagent was standardized by a solution freshly prepared from lemon juice assuming that all of the reducing value (by iodine) of this extract was due to ascorbic acid.

As others have found, the difficulty is to standardize the extraction technique. Discrepancies between different workers and methods have led to much controversy in the literature which cannot be dealt with here. Only a brief statement of the method applied and the results obtained will be attempted.

If potato tissue is extracted for ascorbic acid as directed by BESSEY and KING (5) in 8 per cent. acetic acid, 90 per cent. of the initial activity is lost in a period as short as 20 minutes. Fresh tissue, however, ground in 8 per cent. acetic acid + M/10 NaCN gave a constant ascorbic acid titre during a period of two hours after the extraction and, after standing overnight exposed to air, 84 per cent. of this original activity still remained. If the extraction technique was preceded by vacuum drying for ¾ hours at 100° C. slightly higher (up to 20 per cent. greater) values were obtained than when extraction took place from the fresh tissue in presence of cyanid (table XI). Some (19) in this sort of difference evidence for combined and uncombined ascorbic acid whereas others (18, 38) regard these differences as caused by the more effective inactivation of enzymes in the heated tissue. Since such small differences have not yet been correlated with metabolism, only that method

which gave the best determination of the reduced ascorbic acid was applied (extraction of tissue, vacuum dried, in presence of cyanide). The ascorbic acid exists in washed, living discs *only* in the reduced form. If extracts were made as above, saturated with hydrogen sulphide, and the latter removed in a vacuum in the presence of cyanide, the titrations were the same as for the extract untreated with hydrogen sulphide.

Effect of time on the ascorbic acid content of potato discs in aerated salt solution

In thin potato discs in aerated solutions at 23° C. a progressive *increase* in the content of ascorbic acid occurs (see two series of table XII). This

TABLE XII

Ascorbic acid content of potato discs in aerated salt solutions at 23° C.

Series	Hours	Ascorbic acid per gram fresh weight
		mg.
KCl (0.001 equivalents, per liter)	0.0	0.094
	75.5	0.161
	125.0	0.198
$CaCl_2$ (0.001 equivalents, per liter)	0.0	0.094
	75.5	0.155
	125.0	0.188

occurs in tissue in contact with aerated distilled water at 23° C. and is yet another symptom (there is no reason to believe that it is a cause) of the increased metabolism which, although it can proceed independently of salt absorption, is a prominent feature of the system which is capable of absorp-

TABLE XIII

Effect of aerated salt solutions and time on the content of reduced ascorbic acid and glutathione in potato discs

Composition	External solution. Salt concentration per liter	Sample	Period hours	Ascorbic acid per gram initial fresh weight	Glutathione per gram initial fresh weight
				mg.	*mg.*
......	......	Initial	0.0	0.104	0.064
......	......	Initial	0.0	0.102	0.059
H_2O	......	Final	70.0	0.151	0.059
KCl solution	0.05	Final	70.0	0.163	0.056
$CaCl_2$ solution	0.05	Final	70.0	0.162	0.060
H_2O	......	Final	115.5	0.189	0.071
KCl solution	0.05	Final	115.5	0.199	0.067
$CaCl_2$ solution	0.05	Final	115.5	0.213	0.065

tion. The salts which do affect respiration (KCl which increases it, and $CaCl_2$ which decreases it) have only a little, and barely significant effect on the final, total ascorbic acid content (compare the data for the KCl and $CaCl_2$ series at the same time periods in tables XII and XIII). If—against the weight of evidence—the total ascorbic acid content does include a component which is either combined or in intimate association with the protoplasm it is still possible that the salt treatments affect the combined fraction more significantly than these determinations of total ascorbic acid content indicate.

Effect of time and salt solutions on reduced ascorbic acid and glutathione in potato discs

The simultaneous effects of the salt and aeration treatment upon the glutathione and ascorbic acid content of potato discs was examined (table XIII). Glutathione, to which a special rôle in biological oxidation has been ascribed, is now known to bear a definite relationship to the oxidation of ascorbic acid by the so-called "hexo-oxidase" of Szent-Gyorgyi (41). Hexo-oxidase is a specific enzyme of the expressed juice of cabbage, which Zilva (45) thought was present in apple and which oxidizes ascorbic acid (hexuronic acid) to dehydro-ascorbic acid. According to Hopkins and Morgan (12) the enzyme hexo-oxidase does not oxidise glutathione directly, but in mixtures of glutathione and ascorbic acid the former is oxidized at the rate at which the latter would have disappeared if the glutathione had been absent. In other words the ascorbic acid is oxidized and the glutathione promptly reduces it, so that ascorbic acid behaves like a co-enzyme in the oxidation of glutathione by "hexo-oxidase" and itself remains in the reduced condition.

After the treatments indicated in table XIII the potato discs were vacuum dried and extracted with acetic acid. In the one case (determination of ascorbic acid) the extract was titrated when standardized ascorbic acid reagent (2 : 6 dichlorophenolindophenol) and in the other, for the determination of total reducing value (ascorbic acid + glutathione), with 0.05 N iodine according to the method of Hopkins and Morgan (12).

The data (table XIII) show again that, at 23° C., in thin discs in aerated solutions, the content of ascorbic acid rises in a manner which bears an approximately linear relationship to time. Comparison of tables XII and XIII with figure 3 verifies that the change in the ascorbic acid content of the discs with time does not follow the same course as the respiratory time drift, (*e.g.*, tissue in distilled water) neither is there an outstanding effect which may be attributed to the presence of salts. Glutathione (reduced) contributes only a small part of the total reducing value and the quantity present in the discs does not change significantly when they are exposed to aerated salt solutions.

These data show that the total ascorbic acid content of the living cells is not the factor which directly determines either the browning reaction referred to or the respiratory rate of the cells. The salt effects upon respiration, protein synthesis, and oxidase action in the living cells must either be localized in centers distinct from the milieu in which the ascorbic acid occurs or else the ascorbic acid is protected against oxidation by other unknown substances. The progressive browning of discs with time is not compatible with the continued increase of their reduced ascorbic acid content and, neither in its relation to time nor to salts is the ascorbic acid content of living potato discs affected in a manner similar to respiration. Future work must determine the nature of the substance, or circumstances, which prevents the ascorbic acid in the living cells from being oxidized by the enzyme present. The now familiar rôle of ascorbic acid as a potential carrier of oxidation as, *e.g.*, between hexo-oxidase and glutathione, suggests that it may enable phenolase to activate a much wider range of substances than its appraised specificity would indicate. Hence, although the quantity of ascorbic acid cannot as yet be correlated with the respiration rate, or even the browning reaction, its presence in actively metabolizing potato discs may yet prove to be essential to the processes they carry out.

A possible relationship between the sugar and ascorbic acid content of potato discs is suggested by the fact that the increase of ascorbic acid occurs during a period of starch hydrolysis, and this requires further investigation. Such relationships, however, like the recognition that ascorbic acid is more concentrated in cells which show vital activity, *e.g.*, the apical regions of roots (**26**), or the young shoots on resting potato tubers (**22**), does not yet justify the supposed regulatory rôle of this substance in the metabolic processes which involve oxidation since it does not distinguish between ascorbic acid as a product and as a primary cause of the vital activity in question.

One can only repeat that the facts are that salts (of K and Ca) do influence the metabolism of potato discs. They exert an effect in the cells upon the activity of the enzyme system which catalyzes the oxidation of phenolic compounds and *in vitro* produces oxidative deamination of amino acids; the suggestion is that the latter processes provide the link with the rate of aerobic respiration and protein synthesis. As yet, however, similar effects of salts have not been demonstrated *in vitro* in preparations which lack the organization of the living cells; and therefore the mechanism of the salt and oxygen effects described cannot be ascribed solely to the oxidase system of the cells. It also involves the more intimate part of the living system which, in conjunction with the system which catalyzes oxidation, brings about protein synthesis. A large number of experiments have been made to investigate the effect of potassium and calcium salts on the browning of tissue extracts, on the oxidation of catechol and of ascorbic acid by crude

potato enzyme preparations, and on the autoxidation (catalyzed by copper) of ascorbic acid. It is clear from these that the work must be continued with purified enzyme preparations.

Summary

The theoretical implications of the data have been elaborated in the text. It remains only to summarize and recapitulate the essential results and conclusions.

The variability in the composition of replicate batches of 40 to 60 standard potato discs, cut from a uniform stock of tubers and washed for 24 hours in running tap water, is small and negligible relative to the changes which occur due to metabolism during periods of the order of 72 hours under the conditions conducive to salt absorption (40 to 60 discs in 2 liters of aerated solution at 23° C.).

Since the fresh and dry weight of the discs changes during the course of metabolism the best basis for the calculation of the results is the initial fresh weight of a known number (40 or 60) of standard discs of known weight (30 or 45 gm.).

The application of conductivity methods to the determination of absorbed carbon dioxide is described. By this means the effect of salts on the time drift in the respiration of potato discs has been investigated.

In relatively strong (0.05 equivalent per liter) potassium salt (KCl) the tissue approaches a steady respiratory rate at a level which far exceeds that of tissue in distilled water and, conversely, in a calcium solution ($CaCl_2$) of the same equivalent strength the steady level (attained after 30 hours) is much lower than that for distilled water. The contrast in the effect of potassium and calcium salts is one which reappears in many aspects of the metabolic behavior of the tissue.

The direct measurement of the heat evolved by potato discs during their metabolism under the required conditions is beset with technical difficulties. The over-all change in the caloric value of the discs, as a result of their metabolism, can be measured by the use of standard bomb calorimetry. The discs decrease in calorific value by amounts much in excess of expectations on the basis of their respiration.

A scheme for the analysis of potato discs has been described. This reveals the changes which occur in the dry weight, fresh weight, starch, sugar, soluble nitrogen and protein content of the discs. Two independent methods show that the estimate of protein synthesized is not appreciably affected by the peculiarities of a single method.

Discs of potato in aerated distilled water or potassium salt solutions synthesize protein. The synthesis is greater in relatively strong potassium salts (KCl) than in water and it can be suppressed in relatively strong (0.075 equivalents per liter) calcium solutions ($CaCl_2$ or $CaBr_2$). The nitrogen for synthesis is drawn from the soluble nitrogen fraction.

The soluble nitrogen fraction of dormant potato tubers consists of both amides and amino compounds and only negligible amounts of free ammonia. The total amide contains two components: the one, probably asparagine, which is stable under hot alcoholic extraction and only hydrolyzed by 6N HCl under reflux condenser for 3 hours and the other, similar to glutamine, which is unstable under hot alcoholic extraction and is hydrolyzed by 2 hours at pH 6 to 7. The accompanying changes in the VAN SLYKE amino nitrogen fraction show that the easily hydrolyzable amide groups behave like that in glutamine. Recognizing these complications, methods are described which permit the determination of the stable amide, the "heat unstable amide" and the amino-nitrogen fraction (free from confusion with the amide group of glutamine-like substances). The fractions determined account quántitatively for the soluble nitrogen content of the tissue.

During protein synthesis nitrogen is drawn from both the stable amide fraction and the amino nitrogen fraction. The bulk of the nitrogen converted to protein is derived from amino compounds other than asparagine. Salts (KCl and $CaCl_2$) influence both the total synthesis of protein and the relative utilization of amino acids and other compounds. Potassium salts increase, and calcium salts decrease, the relative utilization of the amino acids.

Under the conditions of active metabolism and protein synthesis the unstable, glutamine-like amide, increases in the tissue. It appears to be a reactive intermediary and its subsequent utilization is accelerated by the salt conditions which stimulate synthesis.

The metabolic processes described above are reflected in changes in the buffer system of potato. Methods suitable for the rapid electrometric titration of potato sap are described. Titration curves show a strong buffering (pH 8.0–10.0) which cannot be attributed to asparagine; it is shown to be due to amino acids. The amino acid buffering decreases as synthesis of protein occurs. The salts which affect synthesis also affect the titration curve of the sap. Simultaneous effects upon that portion of the titration curve in which organic acid radicals are effective suggest that these also diappear during metabolism in distilled water and their utilization is still greater in calcium bromide solutions.

The salt and oxygen treatments (aerated KCl solutions) which stimulate the respiration of potato discs also increase the superficial browning which is due to the oxidation of phenolic compounds by the aerobic oxidase of potato. The converse effect is observed in aerated calcium chloride solutions. It is believed that the parallel effects of salts on the activity of the oxidase and protein synthesis are to be ascribed to the deaminating action of substances (ortho-quinones) which are intermediates in the oxidation of the phenolic compounds.

The bulk of the reducing (by iodine) action of potato extracts is due to

ascorbic acid. Special precautions are described which nullify the effect of the tissue oxidase on the ascorbic acid in extracts and the determination of the reduced form of ascorbic acid in the potato discs is described. During the rapid metabolism which occurs in tissue in aerated distilled water the reduced ascorbic acid content of potato discs increases progressively with time. Neither in its relation to time, nor to salts, does the ascorbic acid content of potato discs appear to be a causal agent in the respiration or the browning reaction of the living cells. The content of reduced glutathione in the tissue is not significantly affected by the conditions conducive to salt absorption or by the nature of the salt supplied. The possible rôle of ascorbic acid as a carrier of oxygen between the phenolase and non-phenolic substrates is recognized.

The outstanding metabolic processes of potato discs under the conditions conducive to salt accumulation are those which are conditioned by oxygen and the nature of the salts supplied and these are mutually inter-related. Of these the synthesis of protein, the utilization of amino-acids and stable amides, the formation of unstable amides, the use of organic acid radicals and the oxidation phenomenon shown by the browning reaction in the living cells, are all linked with the rate of aerobic respiration and must contribute to the metabolic machinery which renders salt accumulation possible. Only future work can tell which, if any, of these diverse processes is more intimately concerned than all the rest.

This paper is based on work which has been in progress since 1933 and which, started in the Division of Plant Nutrition, University of California, has been continued in the Department of Botany, Birkbeck College, University of London. Collaboration with C. PRESTON was confined to the work at Birkbeck College. Our grateful appreciation of the support which the work received from both laboratories is here acknowledged, and our thanks are accorded to Prof. HOAGLAND for reading the proofs. A grant from the publication fund of Birkbeck College assisted the preparation and publication of figures.

The conductivity apparatus used was provided by a grant from the DIXON Fund of the University of London.

The effective continuation of the work at Birkbeck College, upon which this and subsequent papers are based, was made possible by grants for which separate acknowledgment has been made elsewhere.

BIRKBECK COLLEGE
UNIVERSITY OF LONDON

LITERATURE CITED

1. ALBERA, L. Energiemessungen bei *Aspergillus niger* mit helfe eines automatischen Mikro-Kompensations-calorimeters. Rec. des Trav. bot. néer. 29: 47–163. 1932.

2. ARCHBOLD, H. K. Physiological studies in plant nutrition. VII. The rôle of fructosans in the carbohydrate metabolism of the barley plant. I. Materials used and methods of sugar analysis employed. Ann. Bot. N.S. **2**: 183–202. 1938.
3. BARKER. J. Analytic studies in plant respiration. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Part 3. The relation of the respiration to the concentration of sucrose. Proc. Roy. Soc. London B **119**: 453–473. 1936.
4. BAYLISS, L. E. Conductivity method for the determination of carbon dioxide. Biochem. Jour. **21**: 662–664. 1927.
5. BESSEY, O. A., and KING, C. G. The distribution of vitamin C in plant and animal tissues, and its determination. Jour. Biol. Chem. **103**: 687–698. 1933.
6. BROWNE, C. A. A handbook of sugar analysis. London. 1912.
7. DOWNING, A. C., GERARD, R. W., and HILL, A. V. Heat production of nerve. Proc. Roy. Soc. London B **100**: 223–251. 1926.
8. DUNNE, T. C. Plant buffer systems in relation to the absorption of bases by plants. Hilgardia **7**: 207–234. 1932.
9. FIFE, J. M. The physiology of Azotobacter. Unpublished manuscript. Library of Citrus Experimental Station, Riverside, California.
10. GUSTAFSON, F. G. Intramolecular respiration of tomato fruits. Amer. Jour. Bot. **17**: 1011–1027. 1930.
11. HOAGLAND, D. R., and BROYER, T. C. General nature and the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
12. HOPKINS, F. G., and MORGAN, E. J. Some relations between ascorbic acid and glutathione. Biochem. Jour. **30**: 1447–1462. 1936.
13. JOHNSON, S. S., and ZILVA, S. S. The oxidation of l-ascorbic acid by plant enzymes. Biochem. Jour. **31**: 438–453. 1937.
14. KEILIN, D., and MANN, T. Polyphenol oxidase purification, nature and properties. Proc. Roy. Soc. London B **125**: 187–204. 1938.
15. LEACH, W. Researches on plant respiration. IV. The relation between the respiration in air and in nitrogen of certain seeds during germination. (b) Seeds in which carbohydrates constitute the chief food reserve. Proc. Roy. Soc. London B **119**: 507–521. 1936.
16. LEACH, W., and DENT, K. W. Researches on plant respiration. III. The relationship between respiration in air and in nitrogen of certain seeds during germination. (a) Seeds in which fats constitute the chief food reserve. Proc. Roy. Soc. London B **116**: 150–169. 1934.
17. LOOMIS, W. E. A study of the clearing of alcoholic plant extracts. Plant Physiol. **1**: 179–189. 1926.

18. MACK, G. L. The state of ascorbic acid in plant tissues. Nature **138**: 505–506. 1936.
19. MCHENRY, E. W., and GRAHAM, M. L. Estimation of ascorbic acid by titration. Nature **135**: 871–872. 1935.
20. NEWTON, R. G. An improved electrical conductivity method for the estimation of carbon dioxide and other reactive gases. Ann. Bot. **49**: 381–398. 1935.
21. PARIJA, P. Analytic studies in plant respiration. II. The respiration of apples in nitrogen and its relation to respiration in air. Proc. Roy. Soc. London B **103**: 446–490. 1928.
22. PETT, L. B. Changes in the ascorbic acid and glutathione contents of stored and sprouting potatoes. Biochem. Jour. **30**: 1228–1232. 1936.
23. PHILLIS, E., and MASON, T. G. Studies on the transport of carbohydrates in the cotton plant. III. The polar distribution of sugar in the foliage leaf. Ann. Bot. **47**: 585–634. 1933.
24. PREGL, F. Quantitative organic microanalysis. Transl. by FYLEMAN. London. 1930.
25. RANDALL, M., and ROSSINI, F. D. Heat capacities in aqueous salt solutions. Jour. Amer. Chem. Soc. **51**: 323–345. 1929.
26. REID, MARY E. Localization of ascorbic acid in the cowpea plant at different periods of development. Amer. Jour. Bot. **24**: 445–447. 1937.
27. RICHARDS, H. M. The evolution of heat by wounded plants. Ann. Bot. **11**: 29–63. 1897.
28. SAMISCH, R. The measurement of phenolase activity. Jour. Biol. Chem. **110**: 643–654. 1935.
29. SCOTT, W. W. Standard methods of chemical analysis. London. 1922.
30. SMALL, J. Hydrogen-ion concentration in plant cells and tissues. Protoplasma-monographien II. Berlin. 1929.
31. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
32. ————. The absorption and accumulation of solutes by living plant cells. V. Observations upon the effects of time, oxygen, and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.
33. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **4**: 521–544. 1935.
34. ————. Salt accumulation by plants—the rôle of growth and metabolism. Trans. Faraday Soc. **33**: 1006–1023. 1937.
35. ————, BERRY, W. E., and BROYER, T. C. The absorption and

accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 345–366. 1936.

36. ———, Stout, P. R., and Preston, C. The biochemistry of salt absorption by plants. II. The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. (In press.)

37. ———, Wright, R., and Berry, W. E. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water, with observations on surface: volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.

38. Stone, W. Ascorbic acid oxidase and the state of ascorbic acid in vegetable tissues. Biochem. Jour. **31**: 508–512. 1937.

39. Szent-Gyorgyi, A. v. Zellatmung. IV. Über den Oxydationmechanismus der Kartoffeln. Biochem. Zeits. **162**: 399–412. 1925.

40. ———. Observations on the function of peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative. Biochem. Jour. **22**: 1387–1409. 1928.

41. ———. On the function of hexuronic acid in the respiration of the cabbage leaf. Jour. Biol. Chem. **90**: 385–393. 1931.

42. Van der Plank, J. E. The estimation of sugars in the leaf of the mangold (*Beta vulgaris*). Biochem. Jour. **30**: 457–483. 1936.

43. Vickery, H. B., Pucher, G. W., Leavenworth, C. S., Wakeman, A. J., and Nolan, L. S. Chemical investigations of the tobacco plant. V. Chemical changes that occur during growth. Connecticut Agr. Exp. Sta. Bull. 374. 1935.

44. ———, ———, Clark, H. E., Chibnall, A. C., and Westall, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. **29**: 2710–2720. 1935.

45. Zilva, S. S. Reversible enzymatic oxidation of vitamin C. Biochem. Jour. **28**: 663–666. 1934.

ROOT RESISTANCE AS A CAUSE OF DECREASED WATER ABSORPTION BY PLANTS AT LOW TEMPERATURES

PAUL J. KRAMER

(WITH FIVE FIGURES)

Introduction

It is well known that low soil temperatures decrease the absorption of water by plants. Many years ago SACHS (**24**) reported that tobacco and gourd plants growing in moist soil under conditions favoring a low rate of transpiration wilted when the soil was cooled to 3° to 5° C., but recovered when the soil was warmed to 12° to 18° C. Cabbage and turnips were less affected, absorbing enough water at a soil temperature just above freezing to prevent wilting during times of moderate transpiration. A few years later VESQUE (**29**) made further studies using a potometer method in which the roots of *Hedera helix* were immersed in water and cooled to low temperatures. He reported that absorption by transpiring plants decreased as the temperature was decreased although some absorption occurred even at 0.5° C. The most rapid decrease occurred between 15° and 10° C. VESQUE stated that since temperatures higher than 15° C. sometimes resulted in an increase and sometimes in a decrease in absorption, it was impossible to decide as to the real effect.

KOSAROFF (**17**), by the use of potometers, made an extensive investigation of the effects of low temperature on water intake through living root systems and root systems killed by scalding. He found that lowering the temperature from about 20° C. to freezing retarded the absorption of water by living root systems of Phaseolus and Pisum 25 or 30 per cent. but did not decrease the absorption of water through dead root systems. Lowered temperature decreased water intake through cut branches of woody plants, but not as much as through living root systems. KOSAROFF also reported that certain species including *Sinapis alba* and *Chrysanthemum indicum* were able to absorb water from soil at $-1°$ C.; *Chrysanthemum indicum* and Salix absorbed water from ice in which their roots were frozen. Since the claim that absorption occurred was based on recovery of wilted leaves in a humid atmosphere, it might be supposed that recovery was brought about by movement of water from stem to leaves rather than by absorption through the roots. KOSAROFF discounted this explanation, however, since shoots not in water or ice failed to recover their turgor in a saturated atmosphere.

STAHL (**25**) reported guttation from the leaves of oats, barley, wheat, and *Geranium pyrenaicum* with roots in soil cooled to approximately 0° C. This, he believed, indicated that some absorption was occurring even at freezing. DUNCAN and COOKE (**12**) found that the rate of absorption by sugar cane

plants decreased as the water in which their roots were immersed was cooled from 28° to 10° C. They also state that sugar cane plants growing in well watered soil have been observed to wilt upon cooling the soil to about 8° C.

CLEMENTS and MARTIN (7) investigated the effects of soil temperature on the rate of transpiration of *Helianthus annuus*. The rate of transpiration decreased but slightly with decreasing soil temperature from 37° to about 13° C., but decreased rapidly below 13° C., being reduced to one half at about 3° C. The plants begin to wilt at about 4.5° C. and were completely wilted at 1° C., but rapidly recovered when the soil was again warmed. All plants in these experiments were exposed to similar atmospheric conditions.

ARNDT (1) reported that cotton plants exposed to the sun in an unshaded greenhouse wilted at soil temperatures of 17° to 20° C. and plants in solution cultures wilted at 10° to 18° C.

It is probable that, aside from deficient soil moisture, low soil temperature is the most important environmental factor affecting the rate of water absorption. Considerable so-called winter injury, particularly of evergreens, is really injury from desiccation brought about by bright sun and wind which causes excessive transpiration at times when the soil is frozen, or near freezing, so that absorption is too slow to replace the water lost. MICHAELIS (21) has suggested that this is an important factor in determining the tree line in mountains. WHITFIELD (31) and CLEMENTS and MARTIN (7) also believe that the low soil temperatures occurring at high altitude influence plant growth.

TRANSEAU (28) believed that the xeromorphic characteristics of northern bog plants were caused by slow absorption of water resulting from the low temperatures and poor aeration existing in such bogs. Similar anatomical characters could be produced experimentally by growing plants with low soil temperatures, poor aeration, or in dry sandy soil. FIRBAS (13), on the other hand, reported that low temperatures did not interfere with the absorption of water by plants native to German bogs and decided that decreased absorption resulting from low temperatures could not be a cause of their xeric structure. DÖRING (11) studied the effects on absorption of transferring plants in potometers from 20° to 0° C. and found that while the rate was decreased 70 or 80 per cent. in some species it was not decreased at all in other species.

It is not surprising that soil temperature often exerts a marked influence on the rate of water intake since it can affect the process in several different, although more or less related, ways. The more important of the suggested causes for decreased absorption at low temperatures are as follows:

1. Lowering the soil temperature decreases the rate of movement of water from the soil to the absorbing surfaces of the roots. This effect has not been measured directly with root systems, but the writer (19) found the water-supplying capacity of the soil as measured with soil-point cones to be only

one-half to one-third as great at 0° as at 30° C. WILSON (**32**) also found that the water-supplying capacity was decreased at low soil temperatures. This of course would not be a factor in potometer experiments where the roots are in water. CLEMENTS and MARTIN (7) believed it was of little importance in their experiments on plants rooted in soil, but it seems probable that under certain conditions it may be of some importance.

2. Low temperatures retard the elongation of roots. Since the continual extension of root tips into contact with the water films surrounding hitherto untouched soil particles is very important in making the soil moisture available, a decrease or cessation of root elongation will probably decrease the rate of absorption. This would be most important in soils with a moisture content below the field capacity, a condition which commonly exists in the field.

3. The permeability of cells decreases as the temperature is lowered. DELF (9) found that the rate of plasmolysis and hence permeability of the cell membranes of dandelion scapes and onion leaves decreased with decreasing temperatures from 35° to 5° C. A temperature above 35° seemed to have an injurious effect on permeability as the tissue shrank even in water above this temperature. STILES and JØRGENSEN (**27**) studied the rate of absorption of water by carrot and potato tissue and found that it decreased with lowering of temperature from 30° to 10° C. According to STILES (**26**) who has summarized the literature on this subject, permeability to water increases with increasing temperature to at least 30° C., above which the time factor appears. The temperature coefficient for the rate of diffusion through cell membranes was found to be much higher than that for diffusion in aqueous solutions. The causes of the apparent decrease in permeability are at least partly considered in the next two paragraphs.

4. The viscosity of protoplasm and of the colloidal gels in the cell walls is much higher at low temperatures. The increased viscosity probably retards the movement of water across the mass of living cells lying between the soil and the xylem of the roots. WEBER and HOHENEGGER (**30**) found the viscosity of protoplasm in the root cells of Phaseolus seedlings to be about 4 times as great at – 2.0° C. and 3 times as great at 5.0° C. as at room temperature. BĚLEHRÁDEK (**2**) cites other work indicating a general increase in viscosity of protoplasm at low temperatures and suggests that this may slow down diffusion of various substances and cause the high temperature coefficients characteristic of some biological processes. These changes in viscosity are probably concerned in the changes in permeability accompanying changing temperature which were discussed in paragraph 3.

5. The viscosity of water increases as the temperature decreases, being twice as high at 0° as at 25° C. This not only slows down the rate of movement from soil to roots but must considerably decrease the rate of movement through the root cells themselves. It is doubtless an important factor in the low water-supplying capacity of cold soils previously mentioned. The vapor

pressure of water is decreased, falling from 23.75 mm. of mercury at 25° C. to 4.57 mm. at freezing, markedly affecting diffusion and osmotic processes.

6. The physiological activity of the root cells, especially the rate of respiration, is decreased by low temperature. This would be particularly important if the absorption of water is dependent directly or indirectly upon the expenditure of energy by the root cells themselves. HENDERSON (**14**) found a correlation between the rates of respiration and absorption in roots of corn seedlings and suggested that energy needed for water intake is made available by respiration. HEYL (**15**) reviewed the literature on root pressure and presented data indicating that exudation phenomena show a marked positive response to increased temperature. This, together with other facts, led him to conclude that root pressure is probably an electro-osmotic phenomenon dependent on the respiratory activity of the root cells as a source of energy. CRAFTS and BROYER (**8**) have recently advanced an osmotic theory of root pressure which depends on the physiological activity of the cortical cells to maintain a high concentration of solutes in the xylem vessels.

Little definite evidence concerning the relative importance of the various effects of low temperature on water absorption has ever been presented. It seems, however, that in most instances decreased absorption cannot be caused primarily by decreased water-supplying capacity of the soil nor by decreased rate of root extension. Plants with their root systems in well-watered soil and in dilute nutrient solutions or tap water can be caused to wilt in an hour or less by lowering the temperature of the soil or the water to a few degrees above freezing. Root extension and water supplying capacity cannot be limiting factors on absorption under such conditions.

There has been a general tendency to ascribe the reduced absorption of water at low temperatures to decreased physiological activity of the root cells. Decreased temperatures are accompanied by decreased respiration and decreased secretory activity of the living cells, resulting in little or no root pressure at temperatures near freezing. DÖRING (**11**), for example, found that cooling the root systems of several species to 0° C. stopped bleeding or reduced it to a very low value in only five minutes. It has been found, however, that a decrease in secretory activity is inadequate to explain the decrease in absorption. BOONSTRA (**5**) observed that the rate of transpiration of peas was greatly decreased by low temperature. He decided, however, that the decrease could not have been caused by cessation of any pumping action of the root cells because he was unable to demonstrate the existence of root pressure in transpiring pea plants even at 25° C. In experiments previously described (**18**) it was found that sunflower plants grown in a culture solution and placed in potometers filled with tap water at 6° C. wilted badly within a half hour. When the roots were cut off under water the rate of absorption rose to nearly 10 times the previous rate, then fell to a new equilibrium at 2.5 times the rate prior to removal of the roots. The tops of

these plants recovered their turgor within 2 or 3 minutes after removal of their roots and remained unwilted for the duration of the experiments, or more than an hour. These results indicate that the root pressure mechanism was not playing an essential part in absorption; the wilting attendant upon cooling the roots did not result from failure of the roots to "pump" sufficient water into the tops, but from an excessively high resistance to water movement across the tissues into the xylem of the roots themselves. This high resistance must be effective in slowing down water intake regardless of the mechanism involved.

It was suggested by DÖRING (**11**) that since little or no bleeding occurs at low temperatures most of the decrease in water absorption at these temperatures must result from increased resistance to water movement across the living cells of the roots. It has been shown by the writer (**20**) that the living cells (probably principally the cortical cells) across which water passes before it can enter the xylem offer considerable resistance to water movement even at ordinary temperatures. This is probably the principal reason for the lag of absorption behind transpiration which seems to be characteristic of plants even when adequately supplied with water. It seems probable that the resistance to water movement would vary with temperature and would be much greater at low temperatures. Probably the best data on this are from an experiment by BODE (**4**) in which the rate of water movement through sunflower root systems growing in soil and attached to a vacuum pump was found to increase from 10° to 30° C. This seems to indicate that the resistance to water movement through the roots varies with temperature. In view of these facts, an extensive investigation was made of the effect of temperature on the resistance offered by the tissues of the root to water movement.

Methods and results

The sunflower and tomato plants for these experiments were grown in soil in metal containers, or in nutrient solutions, until strong stems and large root systems had developed. This required from four to six weeks. The tops were then removed near the first node and 5 ml. pipettes graduated in 0.05 ml. were attached to the stumps by rubber tubing and sealed with a mixture of paraffin, beeswax, and tallow. Enough water was added to each pipette to bring the meniscus up to the graduations on the pipette and vacuum was applied for a few seconds to remove any air bubbles.

The root systems, in their containers, were then placed in a water bath which could be maintained at any temperature from freezing to 50° C. by refrigerating and heating units. The pipettes were attached to a vacuum pump by rubber tubing and T-tubes and the pressure on the cut stems was reduced to a point where a pressure gradient of 64 cm. of mercury existed from exterior to interior of the root system. This pressure gradient was

maintained for periods of 30 minutes or one hour, depending upon the rate of absorption. By reading the pipettes at the beginning and end of the period the amount of water absorbed was accurately determined. In certain experiments similar groups of plants were prepared, but not attached to the vacuum pump. The rate of exudation at various temperatures was determined from these plants. The results given are averages of determinations on at least six plants.

In the earlier experiments a single group of plants was used for determinations over the entire range of temperatures from near freezing to as high as 40° C. It was suggested that perhaps a longer period of adjustment to a new temperature was needed than was permitted by this method and that the effects of previous exposure to low temperature might affect the behavior at high temperatures. Several experiments were then performed in which the absorption of a group of six sunflower plants was first determined at 25° C. and then at one of the experimental temperatures. These plants were then discarded and the absorption of a second group was determined at 25° C. and at some other experimental temperature. This procedure was repeated for each temperature at which determinations were to

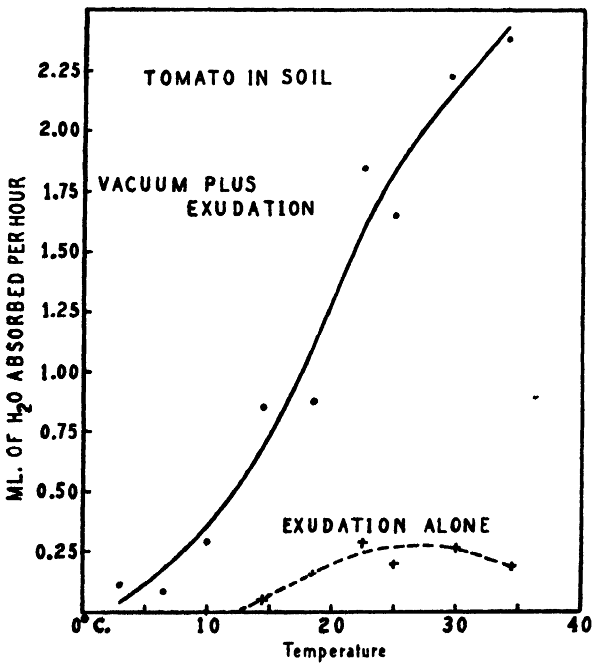


FIG. 1. Water movement in various temperatures through living tomato root systems growing in soil. Each point represents the average of six determinations. The determinations at various temperatures were made on the same plants. The upper curve is for a group of plants attached to a vacuum pump, while the lower curve shows exudation alone.

be made. Thus any one group of plants was exposed to only two temperatures; 25° C., which was approximately that at which it had been grown, and one other temperature which was higher or lower. The rates were then plotted as percentages of the rate at 25° C. The results thus obtained were similar to those obtained when one group was observed at several temperatures. The results of various experiments are much more easily compared, however, when calculated as percentages of the rate at 25° because differences caused by variations in size of root systems are eliminated. The results are shown graphically in figures 1, 2, and 3.

Figure 1 shows the behavior of tomato plants growing in soil at approximately field capacity. It will be noted that the maximum rate of exudation occurred at 22.5° C. and that the rate tended to decrease above this temperature and exudation ceased or become too slow to measure at about 12° C. The highest rate of exudation was only about 15 per cent. of the rate at the same temperature for similar plants attached to a vacuum pump. The rate of water movement at 5° C. through the roots attached to a vacuum pump was only 6 per cent. of the rate of 25° C. Increasing the temperature to 34° C. increased the rate of water movement to 143 per cent. of the rate at 25° C. In a similar experiment with sunflowers the maximum rate of exudation of plants not attached to a vacuum pump occurred at 25° C. and exudation became negligible at about 2.5° C. The highest rate of exudation was only about 17 per cent. of the rate at the same temperature for plants attached to a vacuum pump. BODE (4) also found the maximum rate of exudation of sunflowers at about 25° C. The rate of exudation is quite variable in individual plants, however, and the maximum rate of exudation is sometimes at 35° or 40° C. or higher.

Figure 2 shows the rate of water movement at various temperatures through living sunflower root systems in soil and in water, and through dead root systems in water. All of these root systems were attached to a vacuum pump and maintained under a pressure gradient of 64 cm. of mercury. It will be noted from the graph that the effect of temperature on the rate of water movement through living root systems in moist soil and in water was essentially the same. This perhaps indicates that roots can absorb water as readily from soil near the field capacity as from liquid water. In both instances the rate of movement was much decreased by lowering the temperature. The rate of movement through dead roots was less affected by temperature, indicating that the resistance to flow does not increase as much with lowered temperature in dead roots as in living roots. The lower curve indicates the decreasing viscosity of water with rising temperature plotted as percentages of the reciprocal of the specific viscosity at 25° C. It will be noted that the slope of this curve is approximately the same as that for the rate of water movement through the root systems. The viscosity of water

is about twice as great at 0° C. as at 25° C. and the rate of water movement through the dead roots at 1° C. is about half of the rate at 25° C. This seems to indicate that the viscosity of water may be the principal factor limiting the movement of water through the dead roots. Some additional factor must exist in the living roots which causes their permeability, and hence their resistance to water movement, to change much more with changing temperature than does the resistance to water movement of the dead roots. This additional factor probably is the change in viscosity of the protoplasm and of the colloidal gels of the cell walls. WEBER and HOHENEGGER (30) state that the

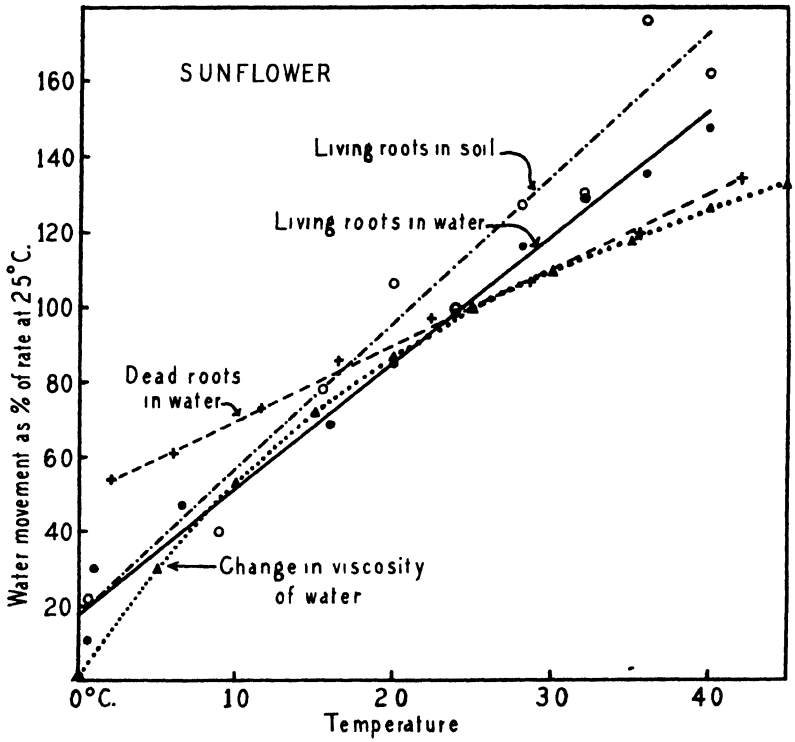


FIG. 2. Water movement through sunflower root systems attached to a vacuum pump. Plants were under vacuum for one hour and a different set of six plants was used for each temperature in all determinations on living roots. The curve for viscosity is the reciprocal of the actual viscosity, plotted as percentages of the value at 25° C. All rates in figures 3 and 4 are plotted as percentages of the rate at 25° C.

viscosity of the protoplasm of Phaseolus epicotyls is about 3 times as great at 5° C. and about 4 times as great at −2.0° C. as at 19° to 22° C. The greater increase in viscosity of protoplasm as compared with that of water probably explains the greater decrease in water movement through living root systems. Killing the cells results in collapse of the protoplasts and dis-

organization of the strands of cytoplasm passing through the cell walls, thus lessening the resistance to flow.

The results of these experiments are similar to those of BODE (4) who measured the rate of water movement at various temperatures from 10° to 30° C. through sunflower root systems attached to a vacuum pump. All the data available indicate a much greater reduction in water movement at low temperatures than the 25 or 30 per cent. reported by KOSAROFF (17). Some variations occur between different groups of plants of the same species, perhaps because of differences in heredity, past treatment, and age. Differences between species might also be expected, but the behavior of sunflower, tomato and privet was essentially the same in these experiments. It would be expected that cotton root systems which, according to ARNDT (1), may wilt at a soil temperature of 15° or 18° C. would show greater reduction of water movement in that temperature range than do sunflowers.

That the resistance to water movement lies chiefly in the protoplasm is indicated by the fact that much more water will pass through a dead root system than passed through the same root system under the same conditions of time, temperature, and pressure while it was alive. The rate of water movement at 25° C. through dead sunflower root systems immersed in water was 3.5 to 6 times the rate through the same root systems while alive. At 1° C. the rate was 5 to 10 times the rate through living root systems. RENNER (23) reported that root resistance was greatly decreased by killing the roots, and the writer (18) has previously reported large increases in water movement after killing the roots.

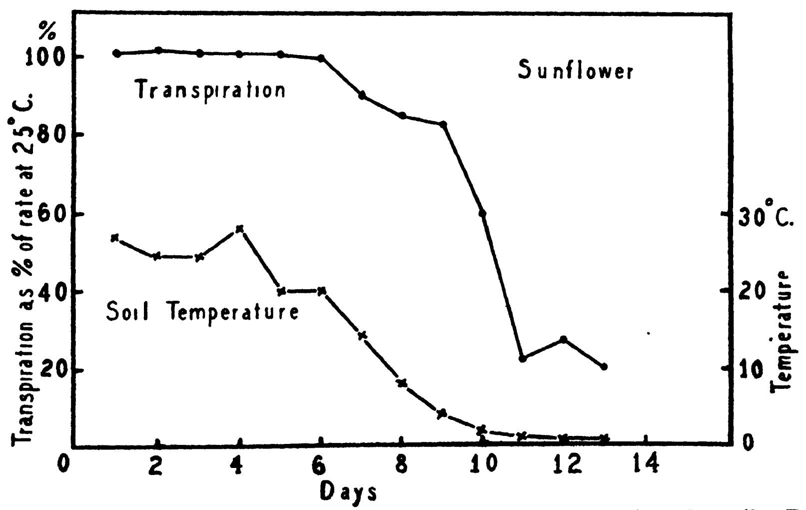


FIG. 3. Effect on transpiration of sunflowers of slowly cooling the soil. Rate of transpiration is plotted as percentages of rate of control group kept in adjacent water bath with soil temperatures of 25° C.

Figure 3 shows the decrease in rate of transpiration of a group of sunflower plants growing in soil which was slowly cooled. This was accomplished by immersing the cans of soil in which the roots were growing in a water bath which was cooled about 4 degrees daily by a refrigeration system. The rate of transpiration is given as percentages of the rate of a group of similar control plants in an adjacent tank kept at about 25° C. The light intensity and air temperatures were essentially the same for the tops of the two groups. It will be noted that at 1° C. the transpiration rate of the sunflowers fell to 20 or 25 per cent. of the rate of the controls at 25° C. This reduction agrees rather closely with the reduction in rate of water movement obtained with root systems attached to vacuum pumps as shown in figure 2. This agreement in results tends to strengthen the belief that data obtained by attaching a vacuum pump to the root system are indicative of what is occurring in the root systems of intact transpiring plants of the same species. A similar experiment was performed on potted plants of privet (*Ligustrum japonicum*). After the soil containing the root systems of these plants had been slowly cooled to about 1° C. the rate of transpiration was about 30 per cent. of the rate of the controls at 25° C.

Discussion

In considering the factors affecting the absorption of water it should be remembered that there appear to be two types of absorption occurring in many plants. These two types of absorption apparently may occur either simultaneously or independently, and their relative importance probably depends on the rate of transpiration and the internal water economy of the plant. RENNER (22) has differentiated these two types as "active" and "passive" absorption, but the writer prefers to term them "physiological" and "physical" absorption.

Active or physiological absorption is some sort of osmotic or secretory process, the mechanism of which cannot be explained fully at present. It is dependent on the presence of living cells in the roots and is responsible for root pressure and exudation from cut stems. Passive or physical absorption is a movement of water across the cortex along a gradient of decreasing vapor pressure from soil to xylem. This pressure gradient results from the decreased pressure or tension on the water conducting system which usually exists in transpiring plants. In these experiments the conditions bringing about physical absorption were partially simulated by attaching the root systems to a vacuum pump.

Previous experiments (20) have shown that at 25° C. there is considerable resistance to the movement of water across the mass of living cells lying between the epidermis and the xylem. The results of the present experiments are believed to indicate that the principal reason for the wilting of

plants whose roots are in cold soil is the reduction in physical or passive absorption caused by increased resistance to water movement across the tissues of the roots. Decreasing the temperature increases the viscosity of water, and to an even greater extent increases the viscosity of the protoplasmic membranes through which the water must pass. As a result of the increased friction less water moves into the roots with a given pressure gradient. The writer prefers to speak of the decreased water movement as being caused by changes in "root resistance" rather than by changes in permeability. Permeability is usually considered with respect to individual membranes. The intake of water is affected not only by changes in the permeability of all the membranes through which the water must pass, but also by changes in the physical properties of the water itself, and the total effect can best be described as root resistance.

The minor part in water intake played by physiological absorption and root pressure phenomena is evident when one observes that the amount of water exuding from cut stems is usually less than 20 per cent. of the amount obtained under a pressure gradient of only 64 cm. of mercury. Such a comparison is open to the criticism that removal of the top decreases the activity of the roots and hence decreases the quantity of exudation and exudation pressure. This effect is probably unimportant during at least the first two or three hours after decapitation as the rate of exudation usually does not begin to decrease for several hours. The resistance of the cortical cells is equally important regardless of whether water is being secreted into the xylem by some root pressure mechanism or moves by mass flow along a gradient of decreasing pressure caused by transpiration.

It appears that Wilson (**32**) is correct in stating that the importance of changes in the viscosity of water have been under-emphasized. Stiles (**26**) points out that the temperature coefficients obtained for movement of water into various living plant tissues are considerably higher than the temperature coefficient for a physical process such as diffusion of a solute into water. Denny (**10**) found the temperature coefficient for the passage of water through non-living seed coats to be higher at a low than at a high temperature. It was also higher than the temperature coefficient for pure diffusion. It is not surprising that the temperature coefficients for movement through such tissues and membranes do not agree with those for diffusion of solutes in water, since the conditions are much more complex in the experiments with plant membranes. The viscosity of the water, the protoplasm, and other colloidal materials of the tissues themselves are increased by lowering the temperature. All of these changes increase the friction or resistance to flow through the membranes and the combined effects will be considerably larger than for diffusion of a solute in water.

According to data of Bigelow (**3**) the rate of movement of water through porcelain and collodion membranes at various temperatures is closely related

to the viscosity of water. In a strict sense the permeability of a porcelain membrane is probably unaffected by small changes in temperature and any change in rate of flow of water is the result of changes in physical properties of the water itself. To discuss the effects of temperature on the permeability of non-living seed coats, porcelain membranes, or dead roots probably misplaces the emphasis. Perhaps, however, we can speak of "membrane resistance" in such instances.

The effects of temperature on the rate of movement of water through various types of non-living membranes is shown in figure 4. The rates of

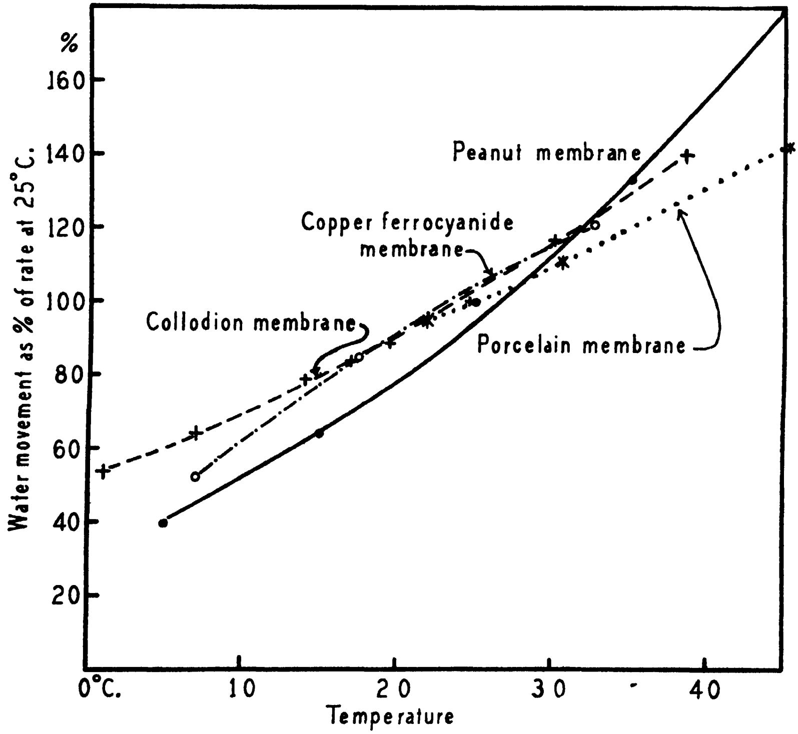


FIG. 4. Effect of temperature on rate of water movement through various types of non-living membranes.

movement are all plotted as percentages of the rates at 25° C. in order to facilitate comparison with the rates of water movement through living and dead root systems plotted in figure 2. The data for peanut seed coats are from DENNY (11). The data for the porcelain and collodion membranes are from BIGELOW (3) and are the rates of flow of water under constant hydrostatic pressure at various temperatures.

Comparison of figures 2 and 4 show that the lines for water movement by both pressure and diffusion through the mechanical membranes have the

same slope as the line for water movement under pressure through dead root systems. The line for movement by diffusion through the peanut seed coat rises somewhat more rapidly with increasing temperature and has a slope similar to that for water movement through the living root systems.

As shown in figure 2, the slopes of these lines resemble the slope of the curve for decreasing viscosity of water with increasing temperature. If viscosity of water were the only factor affecting the rate of movement of water through the membranes at the various temperatures, the lines for rate of movement should be somewhat curved to conform to the curve for the viscosity of water. It is possible that the surprisingly straight lines observed when these diverse data are plotted result from a balancing interaction between the changes in vapor pressure and viscosity that accompany changes in temperatures. Figure 5 shows the vapor pressure of water plotted as percentages of the vapor pressure at 25° C. and the curve for the recip-

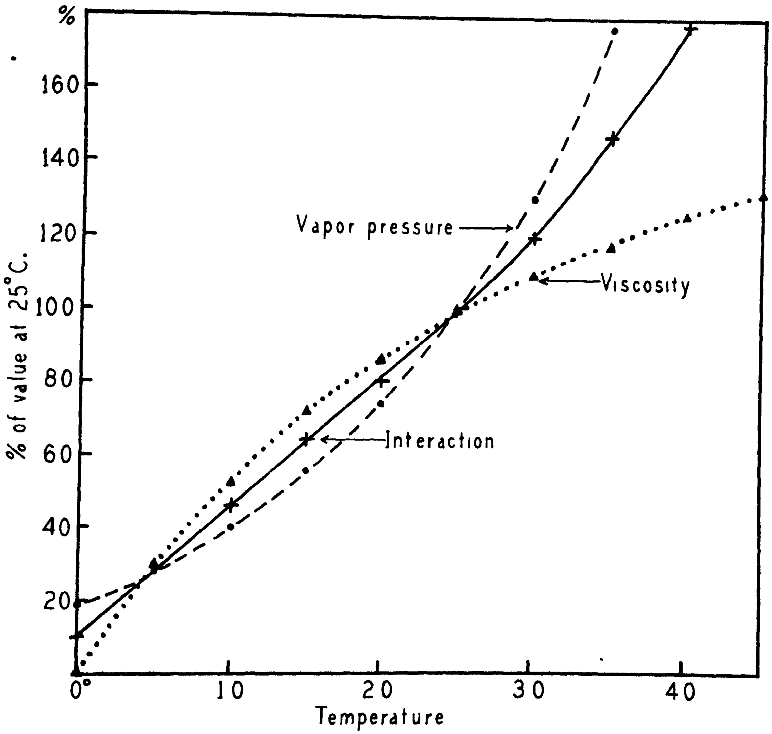


FIG. 5. Changes in viscosity and vapor pressure of water plotted as percentages of values at 25° C. The solid line is drawn equidistant between the two curves showing their possible interaction on water movement.

rocal of the viscosity plotted in the same manner. It will be observed that the line connecting points which are equidistant between the two curves is

practically straight, up to 30° C. Above 30° C. it curves upward because the increase in vapor pressure begins to occur more rapidly than the decrease in viscosity. This suggests that the straight lines obtained for the movement of water are the results of the balancing interaction of viscosity and vapor pressure. The line for water movement by diffusion through peanut seed coats definitely curves upward, especially at 35° and 45° C., as would be expected in a pure diffusion process dependent on a vapor pressure or activity gradient.

Changes in viscosity of water and especially in the viscosity of the protoplasm possibly have some effects on the absorption of minerals. HOAGLAND and BROYER (**16**) report a Q_{10} for salt accumulation by roots of 2.5 to 5.0 No doubt this results largely from the fact that salt accumulation depends on the metabolic activity of the cells. It is likely, however, that if resistance to water movement is four or five times as great at temperatures near freezing as it is at 25° C., then resistance to the movement of ions into cells is also increased by lowered temperature.

While the effects on plant growth of decreased water absorption caused by low soil temperatures have been studied frequently by ecologists and physiologists they possibly have not been considered adequately by agronomists, horticulturists, and others working on problems of plant production. According to investigations previously cited (**1**, **12**), it appears that the absorption of water by cotton and sugar cane plants is checked at temperatures which are not low enough to cause wilting in sunflower or privet plants. BROWN (**6**) has recently published an interesting study of the effects of soil temperature on the growth of Bermuda grass, and Kentucky and Canada bluegrass. When the soil temperature was 21° C. and the air temperature 38° C. the leaves of Bermuda grass wilted, apparently because of inadequate absorption of water; the other two species did not wilt. Absorption of water by Bermuda grass was definitely retarded at soil temperatures of 4.4° and 10° C. while the other two species showed no wilting. It seems possible that the growth of such plants as cotton and Bermuda grass might be hindered by moderately low soil temperatures even though the air temperatures are entirely favorable for growth. One would expect this to be characteristic of most southern species but it is probably unsafe to generalize on this matter. DÖRING (**10**) studied the effect of low temperatures on the water absorption of 57 species having quite different climatic and temperature requirements; he found no consistent relationship between habitat and the extent to which low temperatures checked absorption. The data on cotton and Bermuda grass, however, indicate the possibility that the effects of low soil temperatures on the absorption of water and minerals might limit the growth of plants as effectively as low air temperatures.

Summary

1. The rates of water movement through root systems of tomato and sunflower attached to a vacuum pump were measured at constant pressure over a temperature range from 0° to 40° C. It was found in all experiments that the rate decreased with lower temperatures, both for plants in soil and in water. The rate of movement through sunflower roots at temperatures slightly above freezing averaged about 20 per cent. of the rate through the same roots at 25° C. and the rate at 40° C. averaged about 160 per cent. of the rate at 25° C.

2. The rate of exudation from root systems not attached to a vacuum pump was highest at about 25° C., decreasing with both increased and decreased temperature. The rate became too low to measure at about 12° C. in tomatoes and 2.5° C. in sunflowers. The highest rate of exudation was less than 20 per cent. of the rate obtained with the same or similar plants attached to a vacuum pump under a pressure gradient of 64 cm. of mercury.

3. The rate of movement of water through dead sunflower roots attached to a vácuum pump also decreased with decreasing temperature, but not to the same extent as in living roots. The rate at 0° C. was about 50 per cent. of the rate at 25° C. and the rate at 40° C. was 130 per cent. of the rate at 25° C.

4. It is believed that the decreased rate of movement of water through dead roots at low temperatures is largely caused by the increased viscosity of water itself. In living roots the added effects of decreased permeability, probably resulting from increased viscosity of the protoplasm and of the colloidal gels of the cell walls, causes an even greater resistance to water movement. The resistance to water movement through living roots of sunflower appears to be four or five times as great at temperatures near freezing as at 25° C. At 40° C. the resistance is only a little over half that at 25° C.

5. The principal cause of decreased water absorption by plants at low temperatures appears to be the combined effects of decreased permeability of the root membranes and increased viscosity of water, resulting in increased resistance to water movement across the living cells of the roots. The effects of low temperature in decreasing root extension, root respiration, active absorption and root pressure phenomena are of secondary importance. The increase in root resistance with decreasing temperature probably is effective, however, in slowing down physiological, or active, absorption of water, and possibly the absorption of solutes by roots.

The writer wishes to acknowledge the receipt of financial aid from the Research Council of Duke University, also the valuable suggestions of Dr. H. S. Perry, the aid of T. H. Wetmore in caring for the plants, and the assistance of several students in making the determinations.

Duke University
Durham, North Carolina

LITERATURE CITED

1. ARNDT, C. H. Water absorption in the cotton plants as affected by soil and water temperature. Plant Physiol. **12**: 703–720. 1937.
2. BĚLEHRÁDEK, J. Temperature and living matter. Protoplasma Monograph 8. Berlin, 1935.
3. BIGELOW, S. L. The permeabilities of collodion, gold beaters skin, parchment paper, and porcelain membranes. Jour. Amer. Chem. Soc. **29**: 1675–1692. 1907.
4. BODE, H. R. Beiträge zur Dynamik der Wasserbewegung in den Gefässpflanzen. Jahrb. wiss. Bot. **62**: 92–127. 1923.
5. BOONSTRA, A. E. H. R. Die Bedeutung des Wurzeldrucks für erhöhte Transpiration der Erbsen bei höherer Wurzeltemperatur. Planta **24**: 59–65. 1935.
6. BROWN, E. M. Some effects of temperature on the growth and chemical composition of certain pasture grasses. Missouri Agr. Exp. Sta. Res. Bull. 299. 1939.
7. CLEMENTS, F. E., and MARTIN, E. V. Effect of soil temperature on transpiration in *Helianthus annuus*. Plant Physiol. **9**: 619–630. 1934.
8. CRAFTS, A. S., and BROYER, T. C. Migration of salts and water into xylem of the roots of higher plants. Amer. Jour. Bot. **25**: 529–534. 1938.
9. DELF, E. M. Studies of protoplasmic permeability by measurement of rate of shrinkage of turgid tissues. I. The influence of temperature on the permeability of protoplasm to water. Ann. Bot. **30**: 283–310. 1916.
10. DENNY, F. E. Permeability of certain plant membranes to water. Bot. Gaz. **63**: 373–397. 1917.
11. DÖRING, B. Die Temperaturabhängigkeit der Wasseraufnahme und ihre ökologische Bedeutung. Zeitschr. Bot. **28**: 305–383. 1935.
12. DUNCAN, H. F., and COOK, D. A. A preliminary investigation on the effect of temperature on root absorption of the sugar cane. Hawaiian Planters Rec. **36**: 31–39. 1932.
13. FIRBAS, F. Untersuchungen über den Wasserhaushalt der Hochmoorpflanzen. Jahrb. wiss. Bot. **74**: 459–696. 1931.
14. HENDERSON, L. Relation between root respiration and absorption. Plant Physiol. **9**: 283–300. 1934.
15. HEYL, J. G. Der Einfluss von Aussenfaktoren auf das Bluten der Pflanzen. Planta **20**: 294–353. 1933.
16. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.

17. KOSAROFF, P. Einfluss verschiedener äusserer Factoren auf die Wasseraufnahme der Pflanzen. Inaug. Dissert. Univ. Leipzig. 1897.
18. KRAMER, P. J. The intake of water through dead root systems and its relation to the problem of absorption by transpiring plants. Amer. Jour. Bot. **20**: 481–492. 1933.
19. ———. Effects of soil temperature on the absorption of water by plants. Science n.s. **79**: 371–372. 1934.
20. ———. Root resistance as a cause of the absorption lag. Amer. Jour. Bot. **25**: 110–113. 1938.
21. MICHAELIS, P. Ökologische Studien an der alpinen Baumgrenze. IV. Zur Kenntniss der Winterlichen Wasserhaushaltes. Jahrb. wiss. Bot. **80**: 169–247. 1934.
22. RENNER, O. Die Wasserversorgung der Pflanzen. Handworterb. Naturwiss. **10**: 538–557. 1915.
23. ———. Versuche zur Bestimmung des Filtrationswiderstandes der Wurzeln. Jahrb. wiss. Bot. **70**: 805–838. 1929.
24. SACHS, J. Textbook of botany. Eng. trans. Oxford. 1875.
25. STAHL, E. Zur Physiologie und Biologie der Exkrete. Flora **113**: 1–132. 1919.
26. STILES, W. Permeability. New Phytol. Reprint no. 13. London. 1924.
27. ———, and JØRGENSEN, I. Studies in permeability. V. The swelling of plant tissue in water and its relation to temperature and various dissolved substances. Ann. Bot. **31**: 415–434. 1917.
28. TRANSEAU, E. N. The bogs and bog flora of the Huron River valley. IV. The ecological characteristics of the bog flora and their causes. Bot. Gaz. **41**: 17–42. 1906.
29. VESQUE, J. De l'influence de la température en sol sur l'absorption de l'eau par les racines. Ann. Sci. Nat. Bot. (ser. 6) **6**: 169–201. 1878.
30. WEBER, F., and HOHENEGGER, H. Reversible Viscositätserhöhung des Protoplasmas bei Kälte. Ber. d. bot. Ges. **41**: 198–204. 1923.
31. WHITFIELD, C. J. Ecological aspects of transpiration. II. Pikes Peak and Santa Barbara Regions: edaphic and climatic aspects. Bot. Gaz. **94**: 183–196. 1932.
32. WILSON, J. D. Private communication. 1934.

SEASONAL VARIATIONS IN THE PRODUCTION OF PLANT PIGMENTS

WILLIAM A. BECK AND RICHARD REDMAN

(WITH FOUR FIGURES)

Introduction

The production of the pigments chlorophyll, xanthophyll, and carotene in green plants apparently depends upon the structure of the colloid carrier, the chloroplast (**30**), and upon the activity of the protoplasm in which the chloroplast is immersed. The favorable physico-chemical state has been termed chloroplastinsymplex (**3**). Production of the pigments probably depends upon the organization of the protoplasm and may be termed a vital or physiological process which involves the activity of certain obscure internal factors. It makes little difference in our present study whether it is assumed that these obscure factors are purely physical or chemical, thus involving no other laws than those that are already known to physicists and chemists; or whether, on the other hand, it is assumed that they involve entities that lie beyond our present knowledge of matter and energy. The organization of the protoplasm which is implied in the use of the term "physiological" will hardly escape any careful observer.

It appears that the production of these pigments depends upon certain factors, some of which are internal and others external. The effects produced by certain external factors may be direct, or they may be indirect, in so far as they affect the "organization" of the protoplasm, which itself may be immediately responsible for the formation of pigment.

The effect of some external factors upon the production of pigments has been studied in plants under controlled conditions in our laboratories (**3, 5, 6, 7, 8, 9, 10**). It was found that light favored the production of all three pigments; while it was essential for chlorophyll production, the carotenoid pigments could develop to a limited extent in etiolated seedlings, from reserve materials contained in the seed, without the aid of light. Carotene and xanthophyll probably form a respiratory mechanism for the protoplasm in the seedling (**3**). Carotene may even be important for the early growth of the seedling, functioning as a "sensitizer" (**5, 27, 30**). LAZAR found that carotene (pro-vitamin A) increased the number of roots formed in the cuttings of Impatiens (**16, 17, 18**). That some relationship exists between carotenoid pigments and growth promoters is indicated by the fact that the absorption spectra of these pigments coincide closely with the spectral distribution of the phototropic sensitivity of the Avena coleoptile and the sporangiophores which normally contain these pigments (**11, 12**). PFEFFER (**24**) suggested that the absence of chlorophyll in etiolated plants might be due to some disturbance of the nutrition complex.

The three pigments develop well in the atmosphere of compressed air, at least for a limited period of time, but not in compressed carbon dioxide (**3**, **7**). The development is roughly proportional to the general metabolism. This is in agreement with the notion that external factors influence the development of pigments because they affect the "organization" of the protoplasm. It did not appear probable from our results that slight changes, such as normally occur seasonally in the pressure of the atmosphere, would seriously affect pigment production in plants.

When the illumination was not particularly intense it was found that the chlorophyll produced was proportional to the quantity of light received by the plant (**9**). Apart from a possible temporary initial decline in the carotene present in etiolated plants, (**5**, **10**, **23**) the production of this pigment was also roughly proportional to the quantity of light received when it was not too intense. Xanthophyll production followed the same rule (**7**).

Other investigators show that the wave length of light employed is an important factor in the process of pigment production (**14**, **15**, **25**, **28**). Seasonal variations in the pigment content of leaves might well be expected since the significant factors which influence the production of pigments in plants vary considerably during a year. These factors are the wave length of the radiation and the intensity of the light and heat; it is probable that the intensity of light and the temperature are factors of greater significance than the wave length of light or the moisture conditions.

Accumulation of pigment depends not only upon the amount produced, but also upon the possible destruction of the pigments by such factors as, for example, excessively intense radiation. Such harmful effects may be produced directly or as the result of partial disorganization of the activity of normal internal factors. LUBIMENKO claims that there is an optimum of light in this respect, and that it varies with the species and the age of the plant. The experiments of SHIRLEY (**29**) also point to such an optimum. From this it seems quite natural that shaded plants might well produce more pigments than plants exposed to intense sunlight. The experiments of BECK (**8**) show that this is actually true in certain cases. It is difficult to say if the results obtained by him are entirely due to the intensity of the light, as such, or if the heat factor involved is largely responsible. It is very probable that the heat plays a considerable rôle. HENRICI (**13**) found that the light affected differently the chlorophyll production in different kinds of plants, and also affected differently the chlorophyll production in older and younger leaves. In general, plants with high chlorophyll content show greater variation with change in illumination than do those with low content, which would make it appear as though the light does not act immediately in the production of the pigment.

LUBIMENKO and HUBBENET (**22**) studied the relationship between temperature and chlorophyll production. They concluded that the increase in

production of the pigment in light is conditioned by temperature, because additional synthesis of the precursor of chlorophyll depends upon the temperature. The minimum temperature was found to be between 2° and 4° C., the optimum 26° C., and the maximum 48° C. It is interesting that these values agree roughly with the respective values of the temperature which produces variation in the osmotic value of the cells of assimilating tissues in plants. If it is assumed that the osmotic value of the assimilating tissues is an indicator of the metabolic activity of the tissue (**1**) then it may be stated that the production of pigment present in the tissue is proportional to the metabolic activity induced by the influencing physical factors of the environment, particularly the intensity of light and the temperature. This follows from what was proved to be true for pigment production under controlled conditions and from the effect of light and temperature on the osmotic value of the assimilating tissues (**2**).

After working with external factors under controlled conditions we proceeded to study the seasonal variations of pigment production under normal conditions. Our work extended over a period of almost two years from August 11, 1936, to May 13, 1938. The study of xanthophyll and carotene was discontinued after the completion of the first cycle but the study of chlorophyll was extended through both.

Methods

Dutch Sweet Clover was chosen as our test plant because it did not freeze readily and was abundant in our large open lawn, where no shadows struck the plants and where they were rarely disturbed. Tests were usually made monthly; they were made more frequently, however, when rapid variations of pigment content were indicated.

The older method of extracting the pigment from dried material was not employed since losses through alcoholysis and changes in the chlorophyll are often experienced when using this method. The pigments were extracted by the methods indicated by SCHERTZ (**26**). BECK's device was employed for the quantitative determination of the pigments (**4**). Two hundred leaves (600 lobes) were taken for each test. The green weight was determined before the extraction was made, and the weight of the dry pulp was determined after the extraction was completed. The three pigments, whose qualities are recorded for a given test, were obtained from the same lot of plants.

The light factor in arbitrary units was obtained by multiplying the average of the number of hours of sunshine reported per day for the period by a correction factor. The factor was unity for the period from March to September. For October, November, and January it was two-thirds and for the month of December, one-half. This expression for the light factor

was a fair approximation of what would have been obtained if we had calculated the efficiency of the light per square inch brought by a ray falling perpendicularly upon a horizontal surface, multiplied by the sine of the angle which the actual beam made with the horizontal, and had multiplied the number of hours of sunshine by the figures thus obtained. That our figures were not far from the real values may be seen from the graphs of light intensity and temperature which were taken directly from the record. They are reasonably well in agreement (fig. 2).

Results and discussion

Results are recorded in table I. The highest values recorded for the three pigments were as follows: chlorophyll 43.5 mg., xanthophyll 3.480 mg., carotene 1.173 mg.

TABLE I

PIGMENT CONTENT OF DUTCH SWEET CLOVER PER 200 LEAVES AND THE VALUES OF THE INFLUENCING FACTORS, LIGHT AND TEMPERATURE, FROM AUGUST 11, 1936 TO OCTOBER 20, 1937

DATE	CHLOROPHYLL	XANTHOPHYLL	CAROTENE	LIGHT FACTOR IN ARBITRARY UNITS	AVERAGE TEMPERATURE
	mg.	*mg.*	*mg.*		°*F.*
8–11–36	16.375	2.915	0.680	11.36	77.2
9–14–36	22.000	1.790	0.642	9.60	79.4
10–14–36	23.250	1.598	0.684	4.99	64.8
11–19–36	16.245	1.800	0.461	3.57	47.4
12–14–36	14.200	1.350	0.360	2.60	33.7
1–12–37	12.600	0.906	0.264	2.56	41.0
2– 9–37	9.970	0.954	0.345	2.35	35.8
3– 2–37	7.540	0.954	0.306	5.33	33.9
3–18–37	5.550	0.954	0.303	6.14	36.4
3–31–37	11.100	1.725	0.435	7.32	41.3
4–13–37	15.720	1.725	0.558	6.23	46.8
4–27–37	31.600	3.480	1.173	6.66	57.0
5–13–37	34.200	2.068	0.684	9.24	58.2
5–20–37	43.500			9.46	57.6
6–15–37	23.600	0.940	0.234	14.95	72.4
7– 8–37	26.750	0.895	0.586	13.65	73.4
7–27–47	21.100	0.920	0.586	15.21	76.2
9– 9–37	24.600	1.880	0.684	10.06	76.2
10–20–37	19.300	2.385	0.729	4.91	59.0

The study of pigment ratios proved interesting. The ratios are recorded in table II. The order of the numbers expressing the relative quantities is the same as that usually found in such studies. The amount of chlorophyll, at one time was 98 times as great as that of the carotene, but on an average it was 36.2 times as great. It was 29 times as great as the xanthophyll at one time but on an average only 12.5 times as great. The amount of xanthophyll

TABLE II

PIGMENT RATIOS FOR THE YEAR 1936–1937

DATE	CHLOROPHYLL: XANTHOPHYLL RATIO	CHLOROPHYLL: CAROTENE RATIO	XANTHOPHYLL: CAROTENE RATIO
8–11–36	5.6	24.0	4.2
9–14–36	12.3	34.0	2.7
10–14–36	14.6	34.0	2.3
11–19–36	9.0	35.0	3.9
12–14–36	10.0	39.0	3.8
1–12–37	13.9	47.0	3.4
2– 9–37	10.4	28.0	2.7
3– 2–37	8.0	24.0	3.1
3–18–37	5.8	18.0	3.1
3–31–37	6.4	25.0	3.9
4–13–37	8.9	28.0	3.1
4–27–37	9.0	27.0	2.8
5–13–37	16.5	50.0	3.0
5–20–37			
6–15–37	23.0	98.0	4.0
7– 8–37	29.0	44.0	1.5
7–27–37	22.0	35.5	1.5
9– 9–37	13.1	35.5	2.6
10–20–37	8.0	26.0	3.1
AVERAGE	12.5	36.2	2.9

was never more than 4.2 times as great as that of the carotene and at one time only 1.5 times as great. The average ratio was 2.9.

The regularity with which the ratios change in the different seasons is significant and suggests some relationship of the pigments, if not in their origin or the building of their molecules, at least in their functions. The slight variation of the xanthophyll : carotene ratio is particularly significant. Xanthophyll and carotene appear to be relatively more abundant at those times immediately preceding a growth period; this is in agreement with the notion that carotenoids serve, directly or indirectly, as growth promoters (**3, 5, 16, 17, 18**).

Water makes up the major part of the green weight as was verified from the weight of dry pulp taken at the conclusion of each extraction. From a comparison of the green weight and the prevailing conditions it would appear that relative humidity, soil moisture, temperature, and light influenced the amount of water absorbed. Our results are in agreement with those obtained by URSPRUNG (**31**) for the seasonal variations of suction tension in plants if we take for granted that high suction tension is interpreted as low water content and *vice versa*. URSPRUNG'S data showed that from June, 1925 to June, 1926 the suction force of Bellis for a given month depended on the distribution, as well as the quantity, of precipitation.

In figure 1 is shown graphically the comparison of green weights and

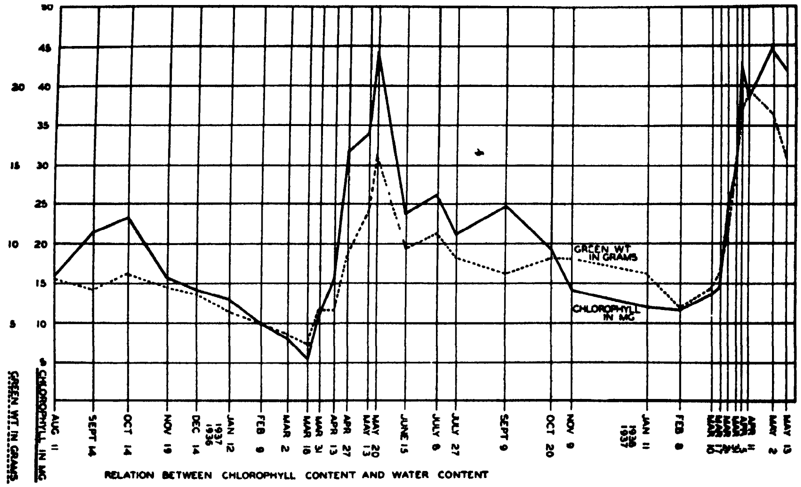


FIG. 1. The relation between chlorophyll content and water content.

chlorophyll yield during the two years. It is evident at once that the general trend of chlorophyll production and water content (assuming that variations in water content are reflected in the variations in green weight) are the same. The predominant factors (light and heat) probably affect both to about the same degree. Less effective factors such as moisture conditions may affect the one somewhat more than the other, producing local deviations in the direction of one graph from that of the other. The chlorophyll production probably depends in a secondary manner upon the water taken up by the plant, but the suction tension of the plant depends upon the moisture of the soil (**31**). A notion of the soil condition may be obtained from the total precipitation for each month.

Comparing the figures for the winter of 1936–1937 with those for the winter of 1937–1938, it must be remembered that the former winter was relatively wet (January, 1937 was the month of the great flood) and the latter relatively dry. The total precipitation values in inches for November, December, and January for the former were 4.09, 3.36, and 14.08, respectively; for the same months of the latter 1.33, 3.54, and 1.46, respectively. The favorable moisture conditions did not favorably affect green weight and pigment production because the temperature was unfavorable in the colder winter of 1936–1937. The spring of 1938 was considerably more wet than the spring of 1937. Precipitation figures for February, March, and April were as follows: 1.22, 1.05, and 2.48 in., respectively, for the same months in 1938, 2.25, 6.41, and 1.69 in., respectively. The favorable temperature permitted the effect of soil moisture to express itself immediately in favor of both the green weight and pigment production (fig. 1). On April 11, 1938 a secondary low is noted for pigment even though the green weight

is at a peak. No such difference is noted for the previous year. The spring of 1937, though relatively dry, had a steady increase in precipitation; in the spring of 1938 the fluctuations of the precipitation were great. In 1938 the temperature dropped from 56° F. through the greater part of March to 42° and 45° F. in April, after which it rose to 63° F. in May. The temperature variation is clearly reflected in the graph for the chlorophyll but not so definitely in the graph for the green weight which was favored somewhat more by the relatively moist soil in the spring of 1938. This clearly shows that while soil moisture does actually favor chlorophyll production it is far less important as a factor than is light or temperature.

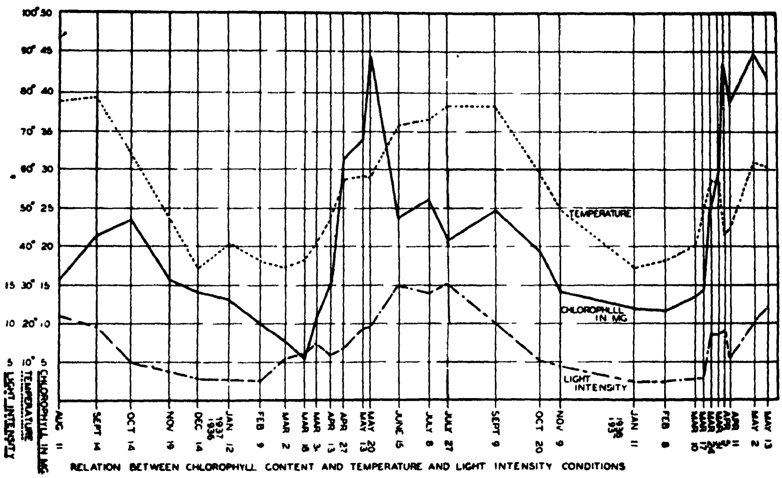


FIG. 2. The relation between chlorophyll content, temperature, and light.

The graphs showing relationships between chlorophyll production and temperature and light are given in figure 2. Similar relationships for xanthophyll and carotene are given in figures 3 and 4.

All three pigments had peaks in the same seasons, spring and fall, and their lowest points in winter and summer (table I and figs. 2, 3, 4). It is particularly interesting that in the spring of 1937 the chlorophyll reached its peak on May 20 but the xanthophyll and carotene had already reached their peak a month before (April 27). In the seedlings studied, under controlled conditions the carotenoids developed in the dark before chlorophyll could develop. This makes the phenomenon of the appearance of carotenoids before chlorophyll more general and gives it more heuristic significance. Since there is some relationship between the carotenoid pigments and growth promoters (**11, 12, 16, 17, 18**) it is not surprising that they should appear in increased quantity in seedlings and mature plants shortly before the time when the plants are developing assimilating tissues in which the chlorophyll

will appear and the plants will avail themselves of those factors in the environment which are particularly favorable for photosynthesis. Factors that favor the development of chlorophyll are evidently considerably less favorable for the development of the carotenoid pigments. The decline in the yield of these pigments later was very marked when the chlorophyll was still increasing in quantity (fall of 1936). The carotenoids were also on the decline when the chlorophyll was in the ascendency. In the fall of 1937 things were not the same since the carotenoids were still increasing even after the chlorophyll had passed its secondary peak on September 9. The summer had been unusual, with drought conditions from June to September. The unusual climatic conditions of the spring of 1937 probably caused the slight difference in the general trend of the three pigments from that of the spring of 1936. In a separate series of experiments it was shown that unusually high heat and drought produce a decline in the carotene yield, at least temporarily (10). In 1937 the peak for the chlorophyll (fig. 2) was reached on May 20 at 43.5 mg. and in 1938, on May 2, at 44.8 mg. The difference is negligible. There was, however, a temporary decline between

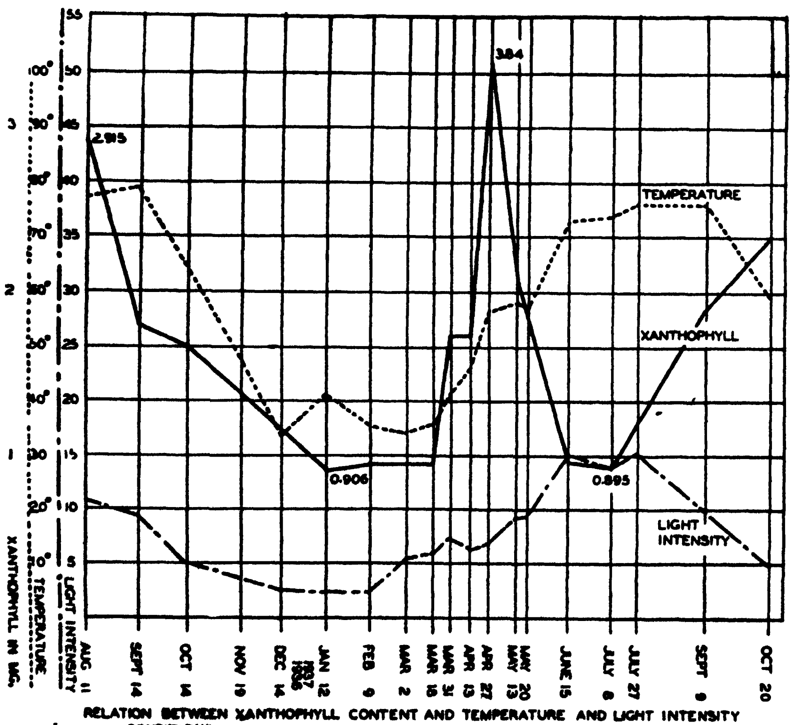


FIG. 3. The relation between xanthophyll content, temperature, and light intensity conditions.

April 5 and the date of the peak which is readily explained by corresponding changes in temperature and light. The predominant effect of the light and temperature throughout the period is evident.

Chlorophyll

The light factor is quite similar to temperature but is predominant. Ten (arbitrary units) is the optimal value of the light factor (fig. 2). This value was obtained on May 20, 1937 at the time of the chlorophyll peak. On June 15, it rose to its peak (15) and the chlorophyll declined; it then returned to peak on July 27 and the chlorophyll again declined to a point even lower than on June 15. This additional decline was undoubtedly caused by the simultaneous rise of the temperature to its peak, which was considerably above the optimum. In spite of the continued high temperature, the chlorophyll rose (July 27 to September 9) because the light factor had returned to the optimum of 10. The chlorophyll could not rise to the value of the primary peak because the temperature was too high. On October 20 the light factor was only 5, the temperature below optimum, and thus the chloro-

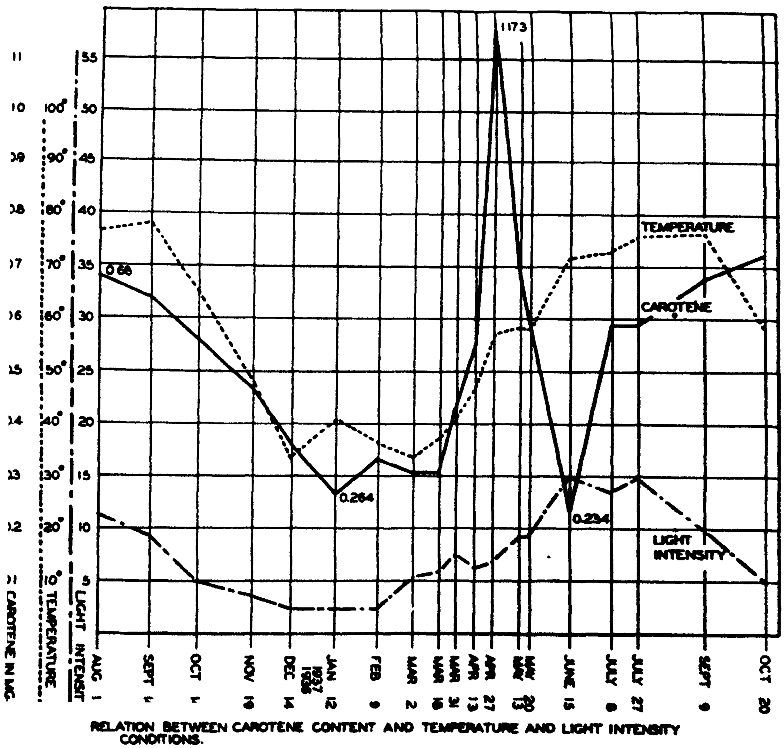


FIG. 4. The relation between carotene content, temperature, and light intensity conditions.

phyll moved into the winter decline. The low was reached February 8, 1938. The light factor and the temperature were about the same at that time, as they were December 14, 1936, on which day the chlorophyll reading was just a little higher. Probably some secondary factors (*e.g.*, soil moisture) were responsible for the difference. The precipitation during the period preceding December 14, 1937 was greater than that during the period preceding February 8, 1938; this accounts satisfactorily for the higher value of the chlorophyll content on December 14, 1937, in spite of the fact that the temperature and light factors were approximately the same on the two dates.

January of 1937 was warmer (flood year) than the January of 1938, but the light factor was about the same; in agreement with these conditions the chlorophyll content was somewhat higher on the first date. The low of March 18, 1937 (7.54 mg.) is considerably below that of February 8, 1938 (11.45 mg.). The drought of February and early part of March, 1937 were probably responsible for this deeper low extending over into March. The rises from low to peak were rapid in both years. The influence of the light factor and temperature is particularly well expressed from April 5 to May 2 in 1938. The chlorophyll yield fell temporarily with light and temperature but rose with them until the peak of the previous year was exceeded by 1.3 mg.

Xanthophyll

The variations of yield of xanthophyll with the variations of light and temperature are shown graphically in figure 3. The peak was reached April 27 at which time the light factor was 6.66 and the temperature was 57° F. Apparently this was the optimum combination of the two factors. It ran quickly to a low as the intense light and heat of summer set in, rising again in fall with the decline of heat and light. The low of January (0.906 mg.) was almost the same as that of summer (0.895 mg.). It is particularly interesting that xanthophyll and chlorophyll do not develop in exactly the same way in response to the radiation factors.

Carotene

The relationships between the development of carotene and light and temperature are shown graphically in figure 4. The peak is reached at the same time as that of xanthophyll, April 27. The optima of the radiation factors are presumably about the same for the development of both pigments. Here also the summer low (July 8) 0.234 mg. is still lower than the winter low (January 12) 2.64 mg. Like the xanthophyll, it was still rising from September 9 to October 20, 1937, while it was falling from August 11 to October 14, 1936. The light factor and temperature conditions were different in the two years and, interpreted from the point of view of the optimum combination, the recorded values are just what might have been expected.

In the fall of 1936 at the times given, the light factors were 11.36 and 9.6 and the temperatures were 77° and 79° F.; in the fall of 1937, however, they were more favorable for carotenoid development, *i.e.*, light factors 10 and 4.9 and temperatures 76° and 59° F.

Summary

1. From the results obtained in studies of pigment yield in plants raised under controlled conditions it appeared probable that seasonal variations would occur in the amount of pigment produced under normal conditions.

2. There were evident and regular variations. The factors may have exercised their influence directly or by affecting the organization of the protoplasm; the latter notion is the more probable. The general trend of variations was the same for all three pigments. A close relationship was revealed between the variations in green weight and the amount of pigment present which suggested that both depended upon the organization of the protoplasm.

3. The three pigments showed a primary peak in their yields in spring and a secondary one in fall. There were corresponding lows in winter and summer. The times of peak and low were about the same for xanthophyll and carotene but the time of peak for the carotenoids preceded that for chlorophyll. The yield of chlorophyll reached its primary peak early in May and the secondary peak in September. The primary low was in the early part of March and the secondary early in July.

4. There was always considerably more chlorophyll present than carotenoid pigment. On an average there was 12.5 times as much chlorophyll as xanthophyll and 36.2 times as much as carotene. There was 2.9 times as much xanthophyll as carotene present on the average. The ratios varied considerably for chlorophyll and the carotenoids but very little for xanthophyll and carotene. In all cases the variations occurred in a remarkably regular manner which suggests a relationship of the pigments, if not in their origin, at least in their functions.

5. The fact that the carotenoids develop vigorously immediately before the growing season is in agreement with the notion that they act, directly or indirectly, as growth promoters.

6. Light and temperature were evidently the factors which most influenced pigment production. Soil moisture played a minor rôle.

7. There is an evident optimum light intensity and also an optimum temperature. Before these optima are reached the production of pigment increases with light and temperature but beyond the optima, production is greatly reduced. These optima are about the same as the corresponding optima for metabolic activity, as deduced from the O_g values of the cell. This suggests that in order to obtain maximum activity and maximum yield

under controlled conditions, the control of light and temperature is as important in summer as it is in winter.

The authors are indebted to the Abbe Meteorological Observatory, Cincinnati, Ohio for their meteorological data and wish to express their thanks for the kind cooperation of its staff.

INSTITUTUM DIVI THOMAE
CINCINNATI, OHIO

LITERATURE CITED

1. BECK, WILLIAM A. Variations in the O_g of plant tissues. Plant Physiol. **6**: 315–323. 1931.
2. ————. The effect of light on the O_g of plant tissues. Protoplasma **23**: 203–210. 1935.
3. ————. The chloroplastinsymplex and the formation of chlorophyll. Protoplasma **27**: 530–533. 1936.
4. ————. A practical device for the rapid quantitative determination of plant pigments. Science n. s. **85**: 368. 1937.
5. ————. Pigments formed in etiolated sunflower seedlings. Protoplasma **28**: 273–282. 1937.
6. ————. Development of carotenoid pigments without the aid of light. Plant Physiol. **12**: 885–886. 1937.
7. ————. The effect of light and atmosphere on the development of plant pigments. Studies of the Institutum Divi Thomae, **1**: 218–244. 1937.
8. ————. The effect of sun and shade on pigment development. Plant Physiol. **13**: 871–872. 1938.
9. ————, REDMAN, R., and SCHROEDER, P. The relation between the development of chlorophyll and the time of exposure to light. Studies of the Institutum Divi Thomae **1**: 245–251. 1937.
10. ————, and SCHROEDER, P. Does irradiation inhibit the development of carotene in sunflower seedlings? Studies of the Institutum Divi Thomae. (In press.)
11. BÜNNING, ERWIN. Phototropismus und Carotinoide. II. Das Carotin der Reizaufnehmezonen von Pilobolus, Phycomyces und Avena. Planta **27**: 148–158. 1937.
12. CASTLE, E. S. The phototropic sensitivity of Phycomyces as related to wave length. Jour. Gen. Physiol. **14**: 701–712. 1931.
13. HENRICI, MARGUERITE. Chlorophyllgehalt und Kohlsäureassimilation bei Alpen- und Ebenenpflanzen. Verhand. Naturforsch. Ges. Basel **30**: 43–136. 1918.
14. INMAN, O. L., ROTHEMUND, P., and KETTERING, C. F. Chlorophyll and chlorophyll development in relation to radiation. Vol. **2**: 1094.

Biological effects of radiation. Edited by B. M. DUGGAR. New York, 1936.
15. JOHNSTON, E. S. The function of radiation in the physiology of plants. II. Smithsonian Misc. Coll. **87**: 14. 1932.
16. LAZAR, O. L'influence du carotène sur la néoformation des racines chez *Impatiens balsamina* L. Compt. Rend. Soc. Biol. **120**: 799–804. 1935.
17. ———. L'influence du carotène sur la néoformation des racines et le developpement de la gemmule chez *Impatiens balsamina* L. Compt. Rend. Soc. Biol. **120**: 1374–1376. 1935.
18. ———. L'influence du carotène sur la néoformation des racines chez *Impatiens balsamina* L. Compt. Rend. Soc. Biol. **121**: 886–889. 1936.
19. LUBIMENKO, W. Sur la sensibilité de l'appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**: 381–415. 1905.
20. ———. La concentration du pigment vert et l'assimilation chlorophyllienne. Rev. Gén. Bot. **20**: 162–177; 217–238; 253–267; 285–297. 1908.
21. ———. Action spécifique de rayons lumineux de diverses couleurs dans la photosynthèse. Compt. Rend. Acad. Sci. (Paris) **177**: 606–608. 1923.
22. LUBIMENKO, V. N., and HUBBENET, E. R. The influence of temperature on the rate of accumulation of chlorophyll in etiolated seedlings. New Phytol. **31**: 26–57. 1932.
23. NORRIS, ROBERT J. Observations on the development of chlorophyll and carotinoid pigment in etiolated plants. Bull. Basic Sci. Res. **5**: 23–33. 1933.
24. PFEFFER, W. Physiology of plants. English trans. by EWART, A. J., Vol. I, 1900. Oxford Univ. Press.
25. SAYRE, J. D. The development of chlorophyll in seedlings in different ranges of wave lengths of light. Plant Physiol. **3**: 71–77. 1928.
26. SCHERTZ, F. M. The extraction and separation of chlorophyll ($\alpha+\beta$) carotin and xanthophyll in fresh green leaves, preliminary to their quantitative determination. Plant Physiol. **3**: 211–216. 1928.
27. ———. The chloroplast pigments, their function and the probable relation of chlorophyll to vitamines. Quart. Rev. Biol. **3**: 459–485. 1928.
28. SCHMIDT, ARNOLD. Die Abhängigkeit der Chlorophylbildung von der Wellenlänge des Lichtes. Beitr. Biol. der Pflanzen. **12**: 269–294. 1914.
29. SHIRLEY, H. L. The influence of light intensity and the quality upon the growth of plants. Amer. Jour. Bot. **15**: 621–622. 1928.

30. STOLL, ARTHUR. Zusammenhänge zwischen der Chemie des Chlorophylls und seiner Funktion in der Photosynthese. Naturwiss. **24**: 53–59. 1936.

31. URSPRUNG, ALFRED. The osmotic quantities of the plant cell. Trans. by BECK, W. A. Proc. Internatl. Congress of Plant Sci. Ithaca **2**: 1081–1094. 1929.

32. WIESNER, J. Untersuchungen über die Beziehungen des Lichtes zum Chlorophyll. Sitzungsber. Kais. Akad. Wiss. Wien **69**: 327–385. 1874.

33. ————. Die Entstehung des Chlorophylls in der Pflanze. Wien, 1877.

EFFECTS OF BORON DEFICIENCY AND EXCESS ON PLANTS

SCOTT V. EATON

(WITH SEVEN FIGURES)

Introduction

There is a large literature in regard to the effects of boron deficiency on plants. Boron is now commonly regarded as an essential element for the growth of plants. Most of the literature demonstrating this fact has appeared during the last 15 years, although certain French investigators, especially BERTRAND, AGULHON and MAZÉ, showed the beneficial effects of boron on the growth of plants much earlier than this. BRENCHLEY (2) reviews this early literature.

In England, BRENCHLEY and WARINGTON (1, 19), and in America, SOMMER and LIPMAN (17) early showed the favorable effects of boron on the growth of various plants. No attempt is made to review the boron literature. MCMURTREY (14) gives a rather recent summary of it. It might be expected that an element needed in such small amounts (0.5 p.p.m. is usually sufficient and may be excessive) would never be lacking in the soil. But cases of deficiency under natural conditions have recently been reported (9, 13).

There is also a large literature dealing with the toxic effects on plants of excessive amounts of boron in natural conditions in the soil and in irrigation water (5, 16), in fertilizers (15), and in nutrient solutions used experimentally (11, 19). Much of the earlier literature in regard to the toxic effects of boron is reviewed by BRENCHLEY (2).

Although there is a large literature on the effects of boron deficiency and excess in the growth of plants, few investigations have used the sunflower. Since the results of the present investigation are rather striking, it is thought worth while to place them on record.

This paper has developed from work done on the effects of sulphur deficiency on the metabolism of plants (7). In this investigation as described below boron deficiency developed; so experiments were performed to determine the proper concentration of boron to use in these sulphur experiments. These experiments were extended into the toxic realm. The methods were essentially the same as those already described (7). Chemicals of C.P. or reagent quality were used, but no attempt was made to further purify them. In the case of the plus-boron cultures, boron was supplied in the form of boric acid. The plants were grown in pure quartz sand, which was given no other treatment than that of flushing it out thoroughly with distilled water before the seed was planted. Somewhat different results might have

been obtained if the chemicals and the sand had been further purified. Most of the data have to do with the sunflower,[1] but in the case of boron deficiency a few data are recorded for the soy bean.

Experimental data

Boron deficiency

Soy bean.—In the spring of 1933, a study was made of the effects of sulphur deficiency on the metabolism of the soy bean (7). The Manchu variety of soy bean was grown in sand culture. About 5½ weeks after the planting of the seed many of the plants had dead tips. Boron in the form of boric acid was applied at a concentration of 0.5 p.p.m. In a short time side shoots were put out. The early symptoms of boron deficiency were not observed, because it was not expected that boron would need to be supplied. In other work, a different variety of soy bean, Wisconsin Early Black, had been grown for 5 weeks without any symptoms of boron deficiency developing (6).

In the autumn of 1936, the Manchu variety of soy bean was grown in sand culture in the greenhouse in order to get more definite symptoms of boron deficiency in the soy bean. The same methods were used as in the 1933 experiment. There were three pots each of plus and minus boron plants. Boron at a concentration of 0.5 p.p.m. was applied twice a week when the pots were flushed out with distilled water. The seed was planted on October 3. By November 18, 46 days from the time of planting, definite symptoms of boron deficiency had developed. The main symptom noticed at this time was a whitening between the veins of the young leaves, the veins themselves appearing much greener than the interveinal areas. By December 8, 66 days from the time of planting, boron deficiency symptoms were quite pronounced. The main symptoms were: dying of the tips, the crinkled character of the upper leaves, the curling downwards of the tips of the young leaves, and the lack of greenness as compared with the plus-boron leaves. The lack of greenness was manifest especially by white lines between the veins of the young leaves, the veins themselves including narrow areas on each side which were much greener. White spots or splotches also developed on the upper leaves. The minus-boron plants were of course much shorter than the plus-boron. Figure 1 gives some idea of the appearance of the leaves; figure 2 compares the height of the plants of the two series. Table I gives the weights of the plants. The plants were harvested on December 8. The data are for 10 plants, but there were 14 plus-boron and 13 minus-boron plants. It is realized that the plants were rather few in

[1] Results quite similar to those recorded in this paper were obtained by Mr. J. C. Frazier in the summer of 1935 in class work under the direction of Dr. Charles A. Shull. He used the water culture method, and showed the effects of both deficiency and excess of boron on the growth of the sunflower.

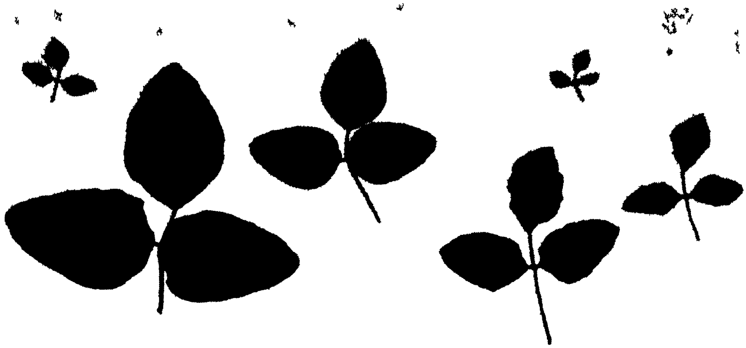


FIG 1 Young leaves of soy bean plants, December 7, 1936, 65 days from planting of seed right, plants receiving no boron, left, plants receiving boron at a concentration of 0 5 p p m

FIG 2 Soy bean plants, December 5, 1936, 63 days from planting of seed showing relative height: right, receiving no boron; left, receiving boron at a concentration of 0.5 p.p.m.

TABLE I

EFFECT OF BORON DEFICIENCY ON GROWTH OF SOY BEAN

Boron	Green weight per 10 plants				
	Leaves	Stems	Tops	Roots	Entire plant
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.0	54.69	41.92	96.61	8.84	105.45
0.5	128.78	73.21	201.99	19.42	221.41

number, but the differences are so great that the results are certainly significant.

It would seem that the soy bean needs a rather small amount of boron for normal growth. Boron at a concentration of 0.5 p.p.m. applied twice a week is certainly sufficient. In fact, in the spring of 1934 (7), normal plants were secured by applying twice a week boron at a concentration of 0.25 p.p.m.

SUNFLOWER.—Considerable preliminary work has been done in a study of the effects of sulphur deficiency on the metabolism of the sunflower, variety Mammoth Russian. One phase of this work was the determination by the triangular method (10) of the best concentration of the salts of the nutrient solution. The plants were grown in sand culture in the spring of 1935 by the same methods as have been described for the soy bean. There were 21 combinations of the salts, and each combination was run in triplicate. Because boron deficiency had developed in the long growing of soy beans, as previously mentioned, it was planned to add boron during the later stages of growth. In less than 4 weeks, however, the upper leaves of many of the plants had assumed an unhealthy appearance. They were crinkled and mottled, and resembled a good deal mosaic infected leaves. In fact, at first the trouble was diagnosed as mosaic, and unsuccessful attempts were made to infect with extracts of these leaves healthy leaves of plants growing in soil. Later it was found that the symptoms were those of boron deficiency but this was not thought of at the time because the trouble had developed so soon and because the symptoms differed somewhat from those of boron deficiency in the soy bean.

Since it was evident before the experiment was completed that the trouble above described was caused by boron deficiency, it was decided to try an experiment with the object of determining the best concentration of boron for optimal growth of the sunflower. The concentrations were extended into the toxic realm in order to determine the nature of the toxic effects of this element on the sunflower. Figure 3 and table II give the concentrations used. The experiment was tried in the autumn of 1935 and was repeated in the spring of 1936. The same methods were used as in the sunflower

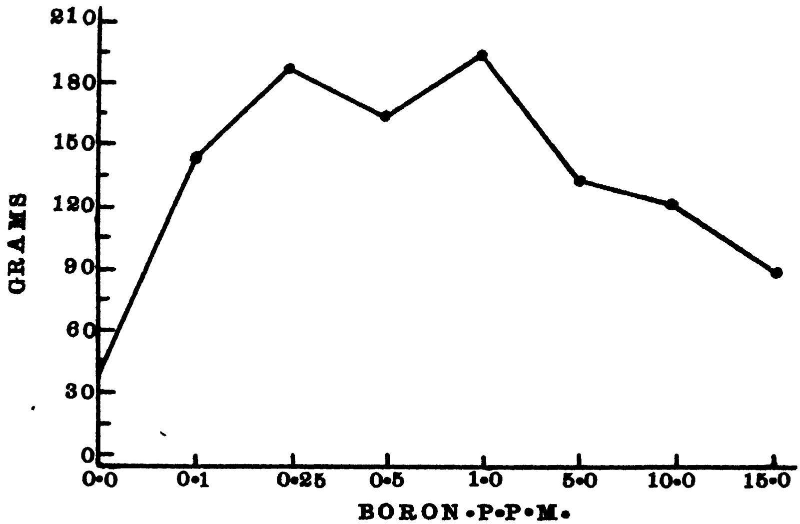


FIG. 3. Green weight per 10 plants of sunflower plants, supplied with various amounts of boron, November 25, 1935, 53 days from planting of seed.

experiment above described. The seed for the 1935 experiment was planted on October 3 and the plants were harvested on November 25; in 1936 the seed was planted on April 3, and harvesting was done on May 22 and 23. Table II gives the weights of the plants of the two experiments, and figure

TABLE II

EFFECT OF VARIATION IN CONCENTRATION OF BORON ON GROWTH OF SUNFLOWER

GREEN WEIGHT PER 10 PLANTS

Boron	Autumn, 1935			Spring, 1936		
	Tops	Roots	Entire plant	Tops	Roots	Entire plant
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.0	38.5	1.25	39.75	439.83	70.0	509.83
0.1	144.0	6.4	150.4	740.66	142.58	883.24
0.25	188.35	9.58	197.93	700.58	152.91	853.49
0.5	165.2	4.5	169.7	759.49	190.83	950.32
1.0	196.0	7.54	203.54	878.63	207.27	1085.9
5.0	135.08	5.16	140.24	512.57	105.21	617.78
10.0	123.09	4.54	127.63	509.41	100.50	609.91
15.0	90.58	3.90	94.48	531.99	86.54	618.53

3 represents graphically the weights of the plants of the 1935 experiment. The weights are for 10 plants, but the actual number of plants ranged from 8 (only one series less than 10) to 12 in the 1935 experiment and from 11 to 18 in 1936. Though the plants are few, the weights differ so decidedly

that they are no doubt significant. Figure 4 compares the height of the plants of the 1936 experiment up to and including the series 5.0 p.p.m.

FIG. 4. Relative height of sunflower plants supplied with various amounts of boron, May 20, 1936, 47 days from planting of seed. (Photograph by DR. J. W. MITCHELL.)

As shown by figures 3 and 4 and table II, boron at a concentration of 0.1 p.p.m., caused a very great increase in the height and weight of the plants. But the greatest growth was in the case of the plants supplied with boron at a concentration of 1.0 p.p.m. This was true in each experiment, though in 1935 the roots of the 0.25 p.p.m. weighed the most. Yet as is mentioned later, 1.00 p.p.m. was definitely toxic. The optimal concentration should, therefore, be regarded as lying between 0.5 p.p.m. and 1.0 p.p.m. The decrease in the weight of the plants in the 0.5 p.p.m. series of the 1935 experiment should not be regarded as significant but attributed to individual variation of plants. The number of plants was rather few.

The growing period of the 1935 experiment was a few days longer than the 1936 experiment; yet, as shown by table II, the 1936 plants made much better growth than the 1935 plants. This is to be expected since the light was better and the days longer in the spring than in the autumn. But because of this greater growth, it would also be expected that boron deficiency would develop sooner, would be more pronounced, and would have a greater effect on the weight of the plants in the spring than in the autumn. The reverse was true. As mentioned later, boron deficiency did not develop as soon in the spring as in the autumn, and table II shows that leaving boron out of the nutrient solution did not have as great an effect on the weight of the plants as compared with the plus-boron series in the spring as in the autumn. Figure 4 shows that the minus-boron plants grew to a considerable height. This no doubt indicates some boron contamination in the

spring experiment at least in the minus-boron series. As stated below, further work seemed to prove that this was true.

Definite symptoms of boron deficiency developed in several plants of the 1935 experiment in two weeks, while in the spring of 1936 no definite symptoms were noticed until 25 days after planting, and then only one or two plants showed the effects. To test out the hypothesis that this later development of the symptoms in the spring than in the autumn indicated some boron contamination at least in the minus-boron series, a few more pots of carefully washed sand were planted with sunflower seed on May 1, 1936. By May 20, definite symptoms of boron deficiency had developed in the minus-boron plants. It is possible that the symptoms were present a few days before this date.

The main symptoms of boron deficiency in the sunflower are as follows. The young leaves are lighter in color. Often they appear silvery white, especially in the lower half, and they may also have a bronze tinge. The lighter color frequently takes the form of a mottling of the leaves, though this is probably more characteristic of older stages of the deficiency. The young leaves often bend downwards against the stem. The crinkled character of the leaves and the curling under of the tips and edges are prominent characters. Sometimes the upper leaves are considerably dissected or lobed. Figures 5 and 6 show some of these characteristics. The plus-boron leaves are not quite normal because of a slight wilting.

FIG. 5. Young leaves of plus- and minus-boron sunflower plants, May 25, 1936, 24 days from planting of seed.

Though we did not determine the optimum concentration for the growth of the soy bean, it is clear from this work that the sunflower requires more boron than does the soy bean. In growing sunflowers it is best to have boron at a concentration of about 0.5 p.p.m. constantly in the nutrient solu-

tion, while soy beans do well with an application twice a week at a concentration of 0.25 to 0.5 p p.m.

FIG. 6. Young leaves of plus- and minus boron sunflower plants, May 21, 1936, 48 days from planting of seed.

BORON TOXICITY

SOY BEAN.—No work was done on the soy bean to determine at what concentration boron has a toxic effect. It might be expected, however, that a plant which needs little boron for optimal growth as compared with some plants, for example, the sunflower, would be very sensitive to an excess of this element. According to some of the literature this is true. COLLINGS (4) found that a concentration of 0.1 to 0.2 p.p.m. in the nutrient solution produced definite symptoms of toxicity on the leaves of the soy bean. It is interesting to note, however, that though this very low concentration was definitely toxic there was increased growth, though not a regular increase, up to a concentration of 2.5 p.p.m.

SUNFLOWER—The minimum concentration of boron at which toxicity developed in this experiment was 1.0 p.p.m. The symptoms were definite at this concentration, though not so very pronounced. As mentioned earlier this is also the concentration giving the greatest growth. As the concentration was increased above 1.0 p.p.m., toxicity, as indicated by the appearance of the leaves, increased and the weights decreased. As shown by table II, this decrease was quite regular in the 1935 experiment, but the plants of the 15 0 p p.m. series of the 1936 experiment weighed more than those of the 10.0 p.p.m. This is no doubt to be accounted for by individual plant variation. The number of plants was rather small.

Boron toxicity in the sunflower is indicated first by a mottling of the tips and edges of the lowest leaves. Later these mottled areas die. In severe cases of injury practically the entire leaf may be affected. Figure 7 gives some idea of the appearance of the leaves. The uninjured leaves do not

FIG. 7. Mature leaves of sunflower plants, showing toxicity of boron at a concentration of 15 p.p.m.

appear quite normal, because they had wilted somewhat before the photograph could be taken. The minimal time required for the toxicity symptoms to develop was not determined, but in the case of the 1936 experiment definite symptoms appeared in 3 weeks.

Discussion

Boron deficiency

The symptoms of boron deficiency are quite definite and characteristic, and once observed are easily detected. It is an element that affects meristematic tissue. The stem tip dies, and the growth of the root tip is also affected, death often resulting. In addition the young leaves become chlorotic, and malformations of various kinds may develop. The effects are similar for different plants, especially as regards the effects on the plant's apices, but differ in detail. For example, in this work the chlorosis of the young soy bean leaves gave them a streaked appearance, the interveinal tissue being affected, though white spots also developed; in the case of the sunflower, the young leaves became silvery white, especially in the lower half, and later there was a mottling of the leaves. The two plants are similar in the dying of the stem tips. These symptoms correspond well with those reported by other investigators (**3**, **8**, **11**, **12**).

Plants, of course, differ decidedly as to the optimal concentration of boron for growth. If one defines optimal concentration as that concentration causing the greatest growth without any indications of toxicity, then the optimal concentration for growth of the sunflower lies between 0.5 and 1.0 p.p.m. The greatest growth was at 1.0 p.p.m., but there were also definite toxicity symptoms at this concentration. The effects of different concentrations were not studied in the case of the soy bean, but since deficiency

symptoms showed up much later in the soy bean than in the sunflower, and since normal soy bean plants were grown by an application twice a week of boron at a concentration of 0.25 to 0.5 p.p.m., while for best growth of the sunflower boron at a concentration of about 0.5 p.p.m. was kept constantly in the nutrient solution, it would seem that the optimal concentration for the growth of the soy bean is less than that for the sunflower.

These results compare fairly well with the results of other investigators. SOMMER and LIPMAN (17) used 0.5 p.p.m. of boron for their controls in their study of the effects of boron deficiency on the growth of the sunflower and other plants, though they did not determine whether this was the optimal concentration. MCHARGUE and CALFEE (11) found 0.7 p.p.m. optimal for the growth of lettuce, and SWANBACK (18) 0.4 p.p.m. for tobacco. On the other hand, COLLINGS (4) found that 0.1 to 0.2 p.p.m. was toxic to the soy bean, although there was stimulation of growth up to a concentration of 2.5 p.p.m. This would seem to support our conclusion given above that the soy bean requires less boron than the sunflower. BRENCHLEY and WARINGTON (3) showed that boron is essential for the normal growth of the soy bean, scarlet runner bean, crimson, red, yellow, and wild white clover, and the broad bean. On the other hand, they found that the pea, barley, and candy tuft could complete their entire life cycle, including flower and fruit development, without any boron in the nutrient solution, as shown by spectroscopic examination. It is admitted, however, that the two groups may differ merely in the amount of boron required, that if still lower amounts of boron could be achieved the second group might merge into the first. The seed of the plants of the latter group may contain enough boron for their complete development. Thus these results do not exclude the possibility that boron may be needed by all higher plants.

The conditions under which the plant is grown affects its sensitiveness to lack of boron. The Manchu soy bean did not develop symptoms of boron deficiency as soon when grown in the autumn as when grown in the spring. Similarly, WARINGTON (20) found in the case of various plants that the deficiency symptoms were less pronounced and their progress retarded under a short day as compared with a long day. MCMURTREY (12) found that aerated cultures of tobacco grew more vigorously and developed more pronounced symptoms of boron deficiency than unaerated.

BORON TOXICITY

While boron deficiency symptoms develop first on the youngest leaves of the plant, boron toxicity shows up first on the mature leaves at the base of the plant. The main symptoms as observed in the sunflower are a mottling of the tips and edges of the lower leaves, later followed by the death of this tissue. In severe cases practically the entire leaf is affected. These

symptoms are similar to those observed by other investigators (**5, 11, 16, 19**).

Plants of course vary decidedly as to their susceptibility to boron excess. In the case of the sunflower, toxicity was first observed at a concentration of 1.0 p.p.m., though if a closer series had been run, this critical toxic concentration might have been lowered somewhat. There was, however, no evidence of toxicity at 0.5 p.p.m. McHARGUE and CALFEE (**11**) found that 0.9 p.p.m. of boron was the lowest concentration toxic to lettuce. On the other hand, the soy bean appears to be injured by a much lower concentration of boron than either the sunflower or lettuce. We did not determine the critical toxic concentration for the soy bean, but since our work indicates that the soy bean requires less boron for optimal growth than does the sunflower, it would be expected that toxicity would show up at a lower concentration. The work of COLLINGS (**4**) indicates that this is true. He found that 0.1 to 0.2 was definitely toxic to the soy bean. Similarly, WARINGTON's (**19**) results showed that boron was much more toxic to some plants than to others. For example, boron at a concentration of about 0.06 p.p.m. was toxic to barley, while in the case of the broad bean it took a concentration of about 1.6 p.p.m. or higher for toxic effects to show up. EATON (**5**) classifies a number of crops into three groups as regards their susceptibility to boron excess: sensitive, semitolerant, and tolerant.

The conditions under which the plant is grown affect the toxicity of boron. For example, BRENCHLEY's (**1**) results showed that plants can stand a higher concentration of toxins in the summer than in the early spring and the late autumn, and WARINGTON (**19**) found that boron was not as toxic to the broadbean when it was grown in the summer as when it was grown in the early autumn or spring, owing perhaps to the more vigorous growth in the summer. In the case of boron deficiency, as pointed out earlier, the more vigorous the growth, the more pronounced are the deficiency symptoms, owing no doubt to the greater need of boron.

As previously stated, the greatest growth may be at concentrations that are clearly toxic as shown by the effects on the leaves. A concentration of boron of 1.0 p.p.m. was definitely toxic to the sunflower; the plants of this series, however, were decidedly larger than those grown at a concentration of 0.5 p.p.m. which was not toxic. In the case of the soy bean, COLLINGS (**4**) found that though 0.1 to 0.2 p.p.m. was definitely toxic there was increased growth though not a regular increase up to 2.5 p.p.m.

The range between toxicity and deficiency of boron is quite narrow. At a concentration of 0.5 p.p.m. there was good growth of the sunflower, but 1.0 p.p.m. proved definitely toxic; as mentioned above, the latter concentration afforded better growth. McHARGUE and CALFEE (**11**) found that 0.7 p.p.m. was optimal for the growth of lettuce and that 0.9 p.p.m. was slightly toxic and 1.2 p.p.m. decidedly so.

Summary

1. The main symptoms of boron deficiency in the sunflower and the soy bean are: a dying of the stem tip, a chlorosis of the young leaves, and certain malformations of these leaves. The main symptom of boron toxicity in the sunflower is a mottling of the older leaves, especially at the tips and edges, followed later by the death of this tissue.

2. Boron deficiency affects the youngest leaves of the plant first; boron toxicity, the oldest. This is well illustrated by the sunflower.

3. Plants differ decidedly as to their susceptibility to boron deficiency and excess. The sunflower requires a rather large amount of boron for optimal growth and is not injured until the concentration becomes quite high; on the other hand, the soy bean requires a rather small amount of boron, but, according to COLLINGS (**4**) is injured by very low concentrations of this element.

4. The conditions under which the plant is grown affects its susceptibility to boron deficiency and excess. The more vigorous the growth the more pronounced are the boron deficiency symptoms, but the less pronounced are the boron toxicity symptoms. The soy bean did not develop boron deficiency so soon when grown in autumn as when grown in the summer; on the other hand, the work of others has shown greater toxicity of boron when the plant is grown in the autumn or spring than when grown in the summer.

5. There may be better growth at concentrations of boron that are definitely toxic. Boron at a concentration of 1.0 p.p.m. was toxic to the sunflower, but the plants of this series were larger than those supplied with less boron.

6. The range between boron deficiency and toxicity is quite narrow. Boron at a concentration of 0.5 p.p.m. afforded good growth of the sunflower, but 1.0 p.p.m. was definitely toxic.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BRENCHLEY, WINIFRED E. On the action of certain compounds of zinc, arsenic, and boron on the growth of plants. Ann. Bot. **28**: 283–301. 1914.
2. ———. Inorganic plant poisons and stimulants. 2nd edition, Cambridge University Press. 1927.
3. ———, and WARINGTON, KATHERINE. The rôle of boron in the growth of plants. Ann. Bot. **41**: 167–187. 1927.
4. COLLINGS, GILBERT H. The influence of boron on the growth of the soybean plant. Soil Sci. **23**: 83–105. 1927.
5. EATON, FRANK M. Boron in soils and irrigation waters and its effect

on plants, with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bull. 448. 1935.

6. EATON, SCOTT V. Effects of variation in day-length and clipping of plants on nodule development and growth of soy bean. Bot. Gaz. **91**: 113–143. 1931.
7. ————. Influence of sulphur deficiency on the metabolism of the soy bean. Bot. Gaz. **97**: 67–100. 1935.
8. HAAS, A. R. C., and KLOTZ, L. J. Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia **5**: 175–197. 1931.
9. KOTILA, J. E., and COONS, G. H. Boron deficiency disease of beets. Facts About Sugar **30**: 373–376. 1935.
10. LIVINGSTON, BURTON E. (Editor). A plan for cooperative research on the salt requirements of representative agricultural plants. 2nd edition. Baltimore. 1919.
11. MCHARGUE, J. S., and CALFEE, R. K. Further evidence that boron is essential for the growth of lettuce. Plant Physiol. **8**: 305–313. 1933.
12. MCMURTREY, J. E. The effect of boron deficiency on the growth of tobacco plants in aerated and unaerated cultures. Jour. Agr. Res. **38**: 371–380. 1929.
13. ————. Boron deficiency in tobacco under field conditions. Jour. Amer. Soc. Agron. **27**: 271–273. 1935.
14. ————. Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. **4**: 183–203. 1938.
15. SCHREINER, OSWALD, BROWN, B. E., SKINNER, J. J., and SHAPOVALOV, M. Crop injury by borax in fertilizers. U. S. Dept. Agr. Dept. Circ. 84. 1920.
16. SCOFIELD, CARL S., and WILCOX, L. V. Boron in irrigation waters. U. S. Dept. Agr. Tech. Bull. 264. 1931.
17. SOMMER, A. L., and LIPMAN, C. B. Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. **1**: 231–249. 1926.
18. SWANBACK, T. ROBERT. The effect of boric acid on the growth of tobacco plants in nutrient solutions. Plant Physiol. **2**: 475–486. 1927.
19. WARINGTON, KATHERINE. The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. **37**: 629–672. 1923.
20. ————. The influence of length of day on the response of plants to boron. Ann. Bot. **47**: 429–457. 1933.

LIGHT-MASS ABSORPTION DURING PHOTOSYNTHESIS

EARLE AUGUSTUS SPESSARD

(WITH FIVE FIGURES)

Introduction

In this paper, and in one to follow it, data will be presented to show that light-mass absorption occurs during photosynthesis. The present paper submits the experimental evidence obtained relative to error, to surface phenomena due to atmospheric conditions during weighing operations, and to the question of CO_2-leakage through glass containers as possible explanations of the weight data.

Experimentation

GENERAL PLAN OF THE EXPERIMENT

LIGHT-ABSORBER.—Plants capable of photosynthesis, and at the same time capable of withstanding the conditions inside an hermetically sealed container, had to be chosen. A preliminary series of extermination tests involving some 5000 mixed cultures of green and blue-green algae were run between 1917 and 1934. Invariably the blue-greens survived the greens. No great difference, however, was observed between the blue-greens and the unicellular greens. These forms were, therefore, chosen as the light-absorbing material.

PROVISION FOR CO_2.—At the time of inoculation and sealing, the plants were in a balanced aquarium condition with protozoa. The source of CO_2 supply for photosynthesis was, therefore, the respiration of both plants and animals, and the decay of dead organisms. This eliminated the necessity for CO_2-leakage through the walls. If, however, the animals should not survive the period of experimentation the necessity for CO_2-leakage would arise. Accordingly a preliminary test to determine this point was made from 1934 to 1935. Samples of a culture containing both plants and animals were sealed within glass tubes. These were examined at the end of 1935, microscopically, through the glass. It was observed that some flagellates were active at the end of that time. While we know, therefore, that the internal supply of CO_2 was present we do not know whether it was adequate.

PROVISION FOR TESTING CO_2-LEAKAGE.—The adequacy of the CO_2 supply not being established, it was, therefore, necessary to determine whether the gas could leak through the walls of the container during the course of the experiment. Solutions of $Ba(OH)_2$ and of KOH were sealed in glass tubes in 1934. At the end of 1935 there appeared to be some indication, precipitates, that CO_2 had leaked through the glass, or that the glass had been attacked with the production of precipitated silicates. In such a case, if

CO_2 diffuses through, so would O_2 and N_2, which would cause weight changes. Additional tests were made, as reported in the following pages.

PROVISION FOR TESTING CO_2-LEAKAGE THROUGH THE WALLS OF THE PLANT FLASK.—CO_2-leakage through the walls of flasks containing KOH might be ascribed to deteriorated walls rather than to defective walls. It would not constitute proof that the plants received their CO_2 supply from the outside. In the case of KOH, the partial vaccuum for that gas would be very high, while in the case of the plant flask, very low, owing to the internal supply. It was decided, however, to run a test in the following manner. During the first half of the experiment one plant flask was kept in normal air like all other flasks. During the second half the same plant flask was placed in CO_2-free air. The flask was placed in a 2-liter desiccator containing 100 ml. of saturated KOH. If the internal supply of CO_2 were inadequate for the requirements of photosynthesis, then the plant flask should gain in weight during the first half, provided leakage occurred, while during the second half, it should not gain in weight.

PROVISION FOR DETERMINING THE EFFECT OF BUOYANCY, TEMPERATURE, AND MOISTURE.—The flasks to be weighed were matched, as nearly as possible, with another in which water was placed, so that the counterbalance weights required would be as low as possible. Since it was impossible to determine accurately the difference in volumes on the two pans, a preliminary series of weighings was made with flasks and with beakers, the volume of one being approximately twice that of the other. These tests showed that the weights varied directly as the temperature when the greater volume was on the left pan. Factors could be worked out, therefore, and corrections for buoyancy be determined, provided variations in atmospheric conditions required their use, and provided also, that it could be determined how the weights followed the temperature. It was decided to avoid factors entirely by weighing the flasks under the same atmospheric conditions during at least the start and finish of the experiment.

PROVISIONS FOR REDUCING ERROR DURING WEIGHING.—It was desired to weigh accurately to within 0.02 mg. The balance, an Ainsworth, Type DLM, had a sensitivity, using the single deflection method, of 5.6. This means that it was possible to secure a delivery of less than 0.02 mg. The rider-width, beam marks, and spaces were all measured and plotted on paper. By this means it was possible to determine seating positions very close to 0.01 mg. An arbitrary rest point of + 6.8 was used at the start and finish. The readings in between were taken at + 5.0. Since, however, the change of rest point is significant when using double deflection, both points were used at the last set of readings. No significant difference was observed. By selecting a constant reading point, there was no difficulty in observing a change of deflection on the reseating of the rider 0.01 mg.

To take readings consistently at this value, time intervals had to be established for various manipulation steps, as follows: a, beam release, 5 min.; b, pan release, 10 min.; c, opening balance, 30 min.; d, washing flasks, 6 hr., and 24 hr. for check; e, brushing flasks, 30 min.; f, rest period for eye, while closed, 2 min. Failure to observe these points results in an error ranging from 0.02 to 0.1 mg.

The rest point was determined both before and after weighing the flasks. If it changed during the weighing, the whole was repeated.

Under certain atmospheric conditions, the sensitivity of the balance becomes suddenly reduced, and may be lost entirely. It is apparently an electrical phenomenon. Its duration may be either one hour or ten days. Its explanation needs investigation. A slight effect is not observable when using double deflection, but under single deflection, the behavior is revealed at once, by the refusal of the return deflection to be complete. Weighing accurately to 0.01 mg. is impossible when this singular behavior is at all apparent. It is partially connected with relative humidity. When that is 64 per cent., the effect is somtimes predictable, but not always.

PREPARATION OF FLASKS AND ARRANGEMENT FOR WEIGHING.—Five K-exax flasks of 25-ml. capacity were selected from the same shipment, cleaned and prepared as follows:

Flask no. 1, plant flask, in which were placed 5 ml. of mud on which blue-green algae were growing with various protozoa.

Flask no. 2, water flask, with 15 ml. sterile distilled water.

Flask no. 3, KOH flask, with 15 ml. N/5 KOH.

Flask no. 4, KOH flask, with 15 ml. N/10 KOH.

Flask no. 5, water flask, with 15 ml. sterile distilled water.

These five flasks were arranged in pairs, so that the plant flask would be weighed against both the N/5 KOH and water flask no. 2, and the KOH flask would likewise be weighed against water flask no. 2, as follows:

Pair 20, left pan, water flask no. 2; right pan, plant flask and weights.

Pair 22, left pan, water flask no. 2; right pan, N/5 KOH flask and weights.

Pair 23, left pan, N/5 KOH flask; right pan, plant flask and weights.

Pair 21, left pan, N/10 KOH flask; right pan, water flask no. 5, and weights. (This pair was considered a check for pair 22.)

The procedure was to weigh the three pairs 20, 22, and 23 during the same weighing period, pair 21 being carried through separately.

In the set-up, 20, 22, and 23 the object is, in each case, on the right pan with the weights. If the object gains in weight, this will be indicated by a loss in the value of the counterbalance weights. The reverse is true in pair 21.

In pairs 20, 22, and 23 the recorded gains in weight will form a cross check as follows: let the plant flask in pair 20 gain n mg. Let the KOH

flask in pair 22 gain *w* mg. Then the plant flask in pair 23 will gain $n-w$ mg. If the reading error is 0.01 mg., the three pairs should check within 0.03 mg., the standard deviation being 0.005 mg.

In addition to the four pairs mentioned, 10 others were prepared to determine the correlation between observed weight-gain and photosynthetic activity. These will be described in full in a later paper.

Weighing data for pairs 20, 22, 23, and for pair 21

The weighing data are given complete as recorded, in tables I, II, III, and IV. The standard deviation, calculated in the usual manner, is given for each period. The values plotted in figures 1, 2, 3, and 4 are the means by periods. No corrections are necessary either for buoyancy or for temperature. The weights follow the temperature slightly, and inversely. A correction factor for temperature having a value of 0.0025 mg. per degree of temperature change, and a buoyancy correction for 1 ml. excess volume on the right pan, in each case, does not significantly alter the results obtained before correction. Since we desire only the beginning and end readings, the atmospheric conditions are so nearly alike that the effect of the difference is less than the reading error.

TABLE I

Weighing data of pair 20.* Left pan, H_2O flask; right pan, plant flask and 13.622 gm. counterbalance weights

Period	Date	Temperature of balance	Pressure in mm. Hg.	Apparent weight	Deviation from mean	Standard deviation
	1938	°C.	*mm.*	*mg.*	*mg.*	*mg*
I	2/22	18.4	771	2.45	0.012	
	23	20.6	770	2.44	0.002	
	3/ 4	24.3	755	2.41	0.028	
	6	21.3	773	2.44	0.002	
	7	19.7	777	2.45	0.012	0.01
Means:	3/ 1	20.9	769	2.438		
II	3/31	22.3	765	2.40	0.003	
	4/ 4	19.8	759	2.40	0.003	
	11	18.6	767	2.40	0.003	
	12	18.2	768	2.41	0.007	0.005
Means:	4/ 6	19.7	765	2.403		
III	6/ 3	26.7	765	2.35	0.00	
	8	26.0	764	2.35	0.00	0.00
Means:	6/ 5	26.3	765	2.35		
IV	10/28	21.0	766	2.29	0.00	
	29	21.8	768	2.29	0.00	0.00
Means:	10/29	21.4	767	2.29		

Note: Plant flask was in CO_2-free atmosphere from 6/8 to 10/28.
Mean time: 243 days.
Gain in weight by plant flask: 0.148 mg.
Annual rate of gain: 0.219 mg.

TABLE II

WEIGHING DATA OF PAIR 23.* LEFT PAN, KOH FLASK; RIGHT, PLANT FLASK AND 13.499 GM. COUNTERBALANCE WEIGHTS

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/22	18.6	771	9.50	0.004	
	23	20.8	770	9.50	0.004	
	3/ 4	20.6	771	9.50	0.004	
	4	24.5	755	9.48	0.016	
	5	24.2	757	9.50	0.004	
	7	20.3	774	9.49	0.006	0.008
Means:	3/ 1	21.5	767	9.496		
II	4/ 9	17.9	766	9.47	0.002	
	9	19.2	767	9.47	0.002	
	10	18.0	770	9.48	0.008	
	11	18.5	766	9.47	0.002	0.005
Means:	4/10	18.4	767	9.472		
III	6/ 3	26.7	765	9.45	0.004	
	3	26.5	765	9.45	0.004	
	7	27.2	760	9.44	0.006	0.005
Means:	6/ 5	26.8	763	9.446		
IV	10/28	23.0	766	9.42	0.00	
	29	20.5	766	9.42	0.00	
	29	21.8	767	9.42	0.00	
	330	19.8	769	9.42	0.00	0.00
Means:	10/29	21.5	767	9.42		

* Note: Plant flask was in CO_2 free atmosphere from 6/8 to 10/28.
Mean time: 243 days.
Gain in weight by plant flask: 0.076 mg.
Annual rate of gain: 0.113 mg.

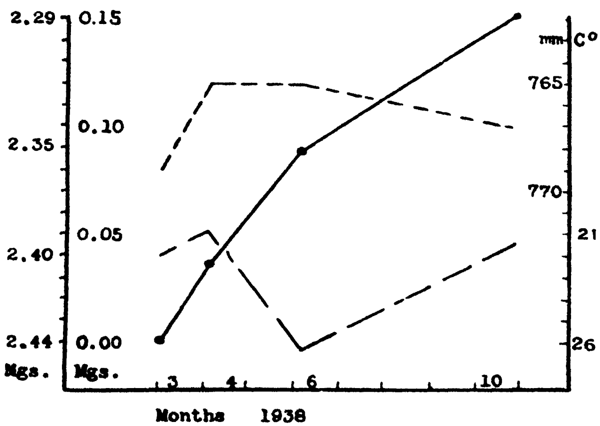


FIG. 1. Pair 20. Curves of mean apparent weights, solid; mean temperature, coarse broken; mean pressure, fine broken lines. All plotted against mean time period. Right milligram column, gain in weight made by the plant flask; left milligram column, values given in table I.

TABLE III

WEIGHING DATA OF PAIR 22.* LEFT PAN, H_2O FLASK; RIGHT PAN, KOH FLASK AND COUNTERBALANCE WEIGHTS, 0.122 GM.

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/23	20.7	760	2.80	0.00	
	24	20.2	772	2.80	0.00	
	3/ 5	24.0	766	2.80	0.00	
	6	21.5	773	2.80	0.00	0.00
Means:	3/ 1	21.6	767	2.80		
II	3/29	20.0	759	2.80	0.002	
	31	22.0	765	2.80	0.002	
	4/ 4	20.0	759	2.78	0.018	
	9	19.1	765	2.80	0.002	0.009
Means:	4/ 3	20.3	762	2.798		
III	6/ 3	26.2	767	2.77	0.000	
	3	26.2	764	2.78	0.01	
	5	27.0	768	2.78	0.01	
	8	26.0	767	2.77	0.00	
	8	26.3	765	2.76	0.02	
	21	27.3	766	2.76	0.02	0.013
Means:	6/11	26.5	766	2.77		
IV	10/28	23.0	766	2.74	0.02	
	29	21.0	768	2.70	0.02	
	29	22.0	767	2.72	0.00	0.015
Means:	10/29	22.0	767	2.72		

* Mean time: 243 days.
Gain in weight by KOH flask: 0.08 mg.

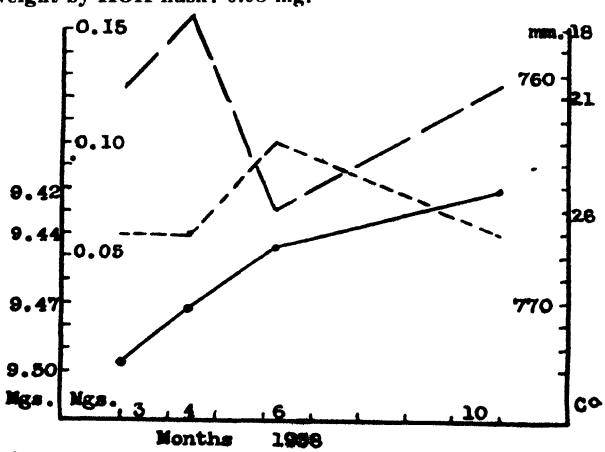


FIG. 2. Pair 23. Legend as in figure 1. In this pair the plant flask of pair 20 (fig. 1) is shown as weighed against N/5 KOH, pair 22 (fig. 3). The plant flask here gained 0.076 mg., while in pair 20, it gained 0.148 mg. This is interpreted as meaning that the KOH flask absorbed CO_2 in sufficient amount to account for the difference of 0.072 mg. The actual gain in weight as shown in pair 22 for the KOH was 0.08 mg. The agreement is very close.

TABLE IV

WEIGHING DATA OF PAIR 21.* N/10 KOH WEIGHED AGAINST WATER. LEFT PAN, KOH; RIGHT PAN, WATER FLASK AND 0.974 GM. COUNTERBALANCE WEIGHTS

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/22	21.0	771	4.52	0.00	
	23	21.0	769	4.52	0.00	
	24	24.0	766	4.52	0.00	0.00
Means:	2/23	22.0	768	4.52		
II	3/ 5	22.0	769	4.52	0.00	
	6	20.2	774	4.52	0.00	0.00
Means:	3/ 6	21.1	771	4.52		
III	6/22	27.9	766	4.55	0.006	
	23	27.3	765	4.56	0.004	
	24	28.0	766	4.56	0.004	0.005
Means:	6/23	27.7	766	4.556		
IV	10/25	21.8	766	4.60	Single reading	

* Mean time: 243 days.
Gain in weight by N/10 KOH flask: 0.08 mg.

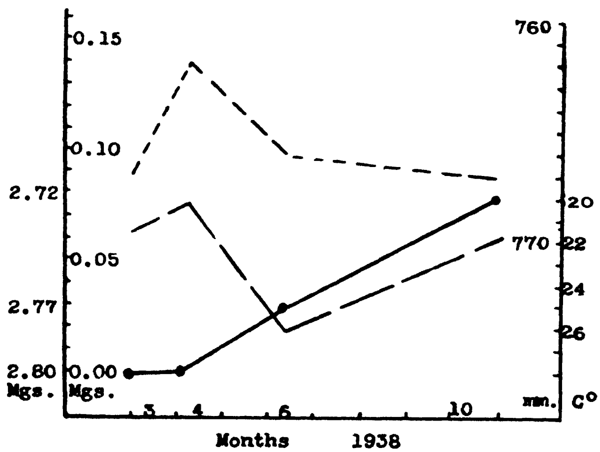


FIG. 3. Pair 22. Legend as in figure 1. The N/5 KOH flask gained 0.08 mg., apparently due to leakage of CO_2 or other gases following deterioration of the glass.

ANALYSIS OF WEIGHING DATA

Without any corrections, the weights for all four points in pairs 20, 22, and 23 check within 0.012 mg. After correcting 20 and 23 as stated above, none being required for pair 22, the weights check within 0.014 mg. This is well within the limits of reading error of 0.03 mg. set previously, in so far as the endpoints, I and IV alone are concerned.

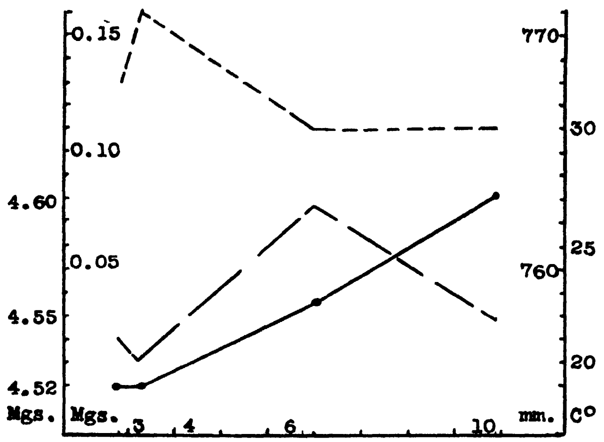


FIG. 4. Pair 21. Legend as in figure 1. N/10 KOH weighed against water. The record is essentially similar to that of the N/5 KOH flask, pair 22, figure 3.

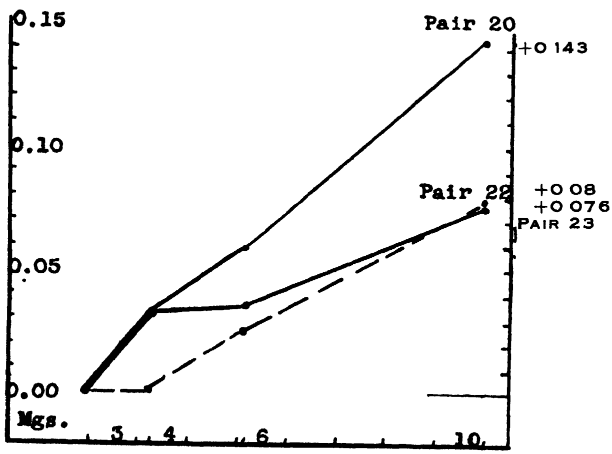


FIG. 5. Apparent weights of pairs 20, upper; 23, middle; and 22, lower, after correcting for buoyancy of 1 ml. for 20, 0.5 ml. for 23, and 0.02 ml. for 22. All points in agreement within 0.03 mg.

Point III is also a critical point when we come to consider whether the rate of weight gain remained the same after June, when the plant flask was placed in CO_2-free atmosphere. A glance at figures 1, 2, and 3 indicates that a slight break occurred in the weight curve of all three pairs at the temperature peak. Correcting for buoyancy alone, figure 5, no doubt partly corrects for temperature also. It is interesting to note that a correction, 1° = 0.005 mg., for pair 20, yields almost the duplicate of the curve

representing 1 ml. buoyancy correction shown in figure 5. For pair 23, the buoyancy correction curve of 0.5 ml. duplicates the temperature curve of $1° = 0.0025$ mg.

The precise differences in volume between the two pans could in no case be determined accurately. To correct for buoyancy it is at least necessary to know on which pan is the greater volume. A number of tests were made to determine this point. In all three pairs, 20, 22 and 23 the volume on the right pan is slightly greater than on the left. Furthermore, this difference is greatest in pair 20, less in pair 23, and least in pair 22. By immersing the flasks of pair 20 in water and calculating the volume of the weights, it was determined that the difference in volume for this pair is approximately 1 ml. From the relative behavior of the two pairs, 20 and 23, during the tests, the difference in volume for pair 23 was taken as about half that of pair 20. That of pair 22 was taken as less than half the latter. Corrections for buoyancy on this determination, yield curves for pairs 20 and 23 consistent with those uncorrected. In figure 5, such curves show that the rate of gain after June was, within error, the same as before.

It appears then, from the data, that the gain in weight observed for the plant flask and for the KOH flasks, is not attributable either to error in weighing or to surface phenomena.

Though the data are not precise on the slight corrections required, after the effect of buoyancy is allowed, it appears probable that the plant flask gained in weight, after transfer to CO_2-free air, at the same rate as before. It should be pointed out that a change in the vitality of the plant after transfer, caused either by a change in light intensity or by increased temperature during the hot summer, might conceivably cause a lowering of the rate of gain. Actually, then, the curve of weights without correction may be as near the true situation as those after correction. In any experiment of this kind, the vital history of the plant is an essential factor. This point will be demonstrated significantly in a succeeding paper.

There remains for consideration, the question of CO_2-leakage through the walls of the plant flask. The fact that the gas has possibly been demonstrated to have leaked through the walls of the KOH flasks is not proof that it leaked through the walls of the plant flask. For in the case of the KOH flask, it is well known that KOH attacks glass. Leakage in those flasks was therefore probably made possible by deteriorated walls, rather than by defective ones. Fortunately, this was anticipated as already stated, by placing the plant flask in CO_2-free air during the last half of the observation period.

From February to April the readings as shown in tables I, II and III, indicate that leakage of CO_2 did not occur, the weights of the KOH flasks remaining the same. During that same period, the weights of the plant flask did show a gain. This is evidence that the gain in weight is not at-

tributable to leakage of CO_2. The proof of this inference lies, however, in the subsequent history of pair 20. From June to Oct., the external supply of CO_2 was cut off from the plant flask. Nevertheless the plant flask continued to gain in weight at approximately the same rate as when the external supply was available. There is therefore no apparent reason for assuming that CO_2 diffused into the plant flask at any time during the experiment.

This suggests another possible source of error. Assume that any of the gases included within the flask at the time of sealing subsequently diffused in part outwardly. A loss of gas would have caused a loss in weight. But, due to its compressibility, the volume of the flask may have therefore been reduced. A reduction in the volume of the plant flask, while the volume of the water flask remained constant, would decrease the buoyancy of the plant flask causing an apparent increase in weight. It would require only a decrease of 0.2 ml. volume to account for the observed annual rate of weight gain, namely, 0.22 mg. This is answered by the fact that the weight of the escaped gas would approximately cancel the loss in buoyancy, assuming for the moment that the flask would be capable of collapsing the amount required.

If, however, the internal pressure decreased gradually without a loss of gas, and if the flexibility of the glass permitted a decrease of one per cent. of the volume of the flask, then the change in buoyancy would account nicely for the observed increase in weight by the plant flask.

Consider first the property of the glass. If we assume 25×10^{-6} as the coefficient of cubic expansion for glass, it would require a lowering of the internal pressure one atmosphere to reduce the volume of the glass 0.0006 ml. This figure is obviously too low to account for the observed increase in weight.

Consider next the volume change in the flask due to the fact that glass is flexible. As a rubber balloon may be inflated or deflated by varying the internal gas pressure, so may we assume changes in a sealed glass flask. So far I have not been able to secure any tables or other data on the degree of volume changes in bulbs or sealed glass vessels. It is obvious that such objects must change in volume to a small extent with variations in internal pressure. But I am assuming that such changes are much less than one per cent. of volume, the amount required to account for the observed increase in weight reported here.

We come finally to the probability of a decrease in the internal volume of gas. Since the immediate question is being considered independently of the question of leakage, the rare gases are ruled out because they are inert. Assume for the moment that for some reason they all leaked out of the flask, even though the percentage of argon exceeds that of CO_2, the total loss of

the rare gases would account for 0.02 ml., assuming further that the glass was flexible enough to take up that volume, and that the decrease in pressure permitted such a change in volume.

At the time of sealing, there was not more than 0.006 ml. of CO_2 in the flask. A thorough depletion of this gas by changing it to solid or liquid compounds within the flask, would therefore not account for the required volume change of 0.2 ml.

The case for O_2 is somewhat different. The excess of O_2 resulting from the process of photosynthesis would be taken up by CO_2 consumption. The respiration of the plants, however, might have given a respiratory quotient less than unity due to the fact that the food storage products are largely fats. If so, there would have resulted an excess pressure of O_2, resulting in an increase in flask volume, resulting in turn in a decrease in weight due to a change in buoyancy under the conditions mentioned earlier. If we consider that animals enclosed at the time of sealing, depleted this excess of O_2 even below the normal volume, such depletion is balanced by the CO_2 given off by the animals during their respiration.

There remains for consideration the gas, nitrogen. It is possible to assume that nitrogen fixing bacteria were included in the culture at the time of sealing. They would take N out of the gaseous state into a solid state, thereby reducing the internal pressure. If complete, the internal gas volume would have been reduced approximately 16 ml. and the internal pressure lowered perhaps 0.7 atmosphere. Assuming that the glass was flexible enough to collapse one per cent. of its volume, this decrease in internal pressure would be sufficient to permit the change. But there is an objection to this interpretation. The plants in all probability used up the nitrogen compounds in their metabolic processes, restoring the N to the internal atmosphere.

It is impossible to decide without chemical analysis of the flask contents at the beginning and end of the experiment, whether the normal nitrogen cycle occured or not. Obviously such an analysis is an impossibility. The impossibility of determining a point in question does not validate data, of course, but rather does it emphasize incompleteness in the experimental arrangement. In other words, while the N question has been answered theoretically, the question lacks exact experimental confirmation.

Concluding remarks

It has been reported in this paper that green plants gain in weight while insulated from their material environment. In the second part which is to follow, data will be presented on 10 other cases, showing that such weight gain is to be correlated with the process of photosynthesis only. The inference will there be drawn to the effect that the gains in weight observed are

explainable only on the assumption that light is absorbed as mass during photosynthesis.

This brings us at once into conflict with Einstein's equation $E = M C^2$. This equation sets the mass limits obtainable through such an experiment as this. The values obtained in the experiment, are, according to various calculations given me, something like three decimal points too high. The equation itself has been verified experimentally from so many independent lines of approach that we are forced to seek an explanation of the discrepancy between the limits set and the values obtained in the present experiment. There are two alternatives. First, the present data are the result of some error or are explainable on some other grounds than the one suggested. Second, an unknown factor in light absorption during photosynthesis precludes a proper application of the equation to the problem.

It is our intention to reexplore the first alternative.

HENDRIX COLLEGE
CONWAY, ARKANSAS

DETERMINATION OF MINERAL ELEMENTS IN PLANT TISSUE[1]

Introduction

The response to the first supplement of methods for the chemical analysis of plant tissue has convinced the committee that such treatment of its function should be undertaken only at relatively long intervals. Only as major improvements in procedure arise is substantial service through this channel likely to be feasible. With the emphasis already placed upon the major groups of organic constituents, *viz.*, carbohydrates, nitrogen-containing compounds, and lipids (SANDO, **26**) there has seemed to remain most obviously for consideration the so-termed inorganic elements.

Current interest in the physiological functions of the rarer mineral elements certainly seems to justify consideration of the more pertinent members of the inorganic group. In view of the rapidly expanding information concerning the occurrence of elements of this group in proteins, and their highly essential rôles in the regulation of enzyme action, the artificiality of attempting to limit their functions by use of the term "inorganic" becomes apparent. The subject here treated is the common meeting ground of students in the fields of both nutrition and metabolism. In the matter of testing for deficiency of rarer mineral elements it is possible that the biological test (plant response) will prove to be of considerable importance in relation to some of the extremely low concentrations involved, as suggested by MCMURTREY (**23**). Nevertheless, it does not appear at present that this diagnostic method will generally supercede determination of the elements in question but rather that it may be expanded and confirmed by the latter.

In passing, it may be advantageous to scan the issue of development in chemical methods of analysis. Perusal of the files of the analytical edition of Industrial and Engineering Chemistry tends to convey the impression of impermanence of results in this field. The considerable sequence of papers concerning some types of determination may be recognized, however, as inspired in part by the desire for absolute accuracy and the individualism incident to this end. For the usual requirement of comparative data on biological samples it appears that extreme accuracy may be sacrificed in favor of other qualifications, such as simplicity and speed. These procedures which are reasonably accurate (variable within a range of 5 to 10 per cent.), suffice for numerous biochemical investigations. In view of the general accessibility of sources of information here cited it has seemed inadvisable to quote procedures in detail. A noteworthy development in the adoption of chemical methods by plant physiologists is the substantial inclusion of these in laboratory manuals (**21**).

[1] Prepared by the committee on chemical methods, Dr. W. E. TOTTINGHAM, chairman.

Methods for the most abundant elements

Procedures for determining these constituents as given in Methods of Analysis (**2**) appear in the main to be as acceptable as any thus far developed. It should be known that critical examination of such procedures, and some issues in process of development, are published periodically in the Journal of the Official Agricultural Chemists. In view of the small proportions of mineral elements to be found in many types of tissue, it is advantageous to conserve material by applying semi-micro technique. The advantage of this form of procedure has been recognized to an increasing extent by the A.O.A.C.

Aluminum

In agricultural practice it was formerly customary to derive the value of this constituent in plant tissue from the combined precipitate of phosphates of iron and aluminum (**2**, p. 122). Aluminum was formerly determined indirectly by use of the value found for iron, but this element is now dealt with directly. Both colorimetric and spectrographic procedures are available for this purpose, the former apparently being more sensitive. A microcolorimetric method is presented tentatively by the A.O.A.C. (**2**, p. 122). The colorimetric procedure of Lundell and Knowles (**22**) might prove adaptable to plant analysis. It is sensitive to less than 0.1 mg.

Calcium

The micro-procedure of the A.O.A.C. (**2**, p. 123) by way of the oxalate should be generally acceptable. Phillips, Smith, and Dearborn (**25**) have suggested that a modification be applied to the usual procedure for removing silica. This involves fusion of the ash with Na_2CO_3, followed by liberation and evaporative dehydration of silicic acid. Titration of the liberated oxalic acid with $KMnO_4$ appears to be generally satisfactory, although Ellis (**9**) has introduced cerium as the oxidant. In back titration of the excess ceric compound, he reports a sharp end point.

Chlorine

The volatility of this element from some of its compounds has been recognized (Fraps, **13**) and is avoided by preparatory impregnation of the sample with Na_2CO_3 before ignition. The solution recovered from the ash can then be treated with excess of $AgNO_3$ and back titrated with KCNS, as advocated by the A.O.A.C. (**2**, p. 131). Direct titration with $AgNO_3$ using K_2CrO_4 as indicator does not seem to be feasible here as it is in water analysis. Wilkens (**32**) has developed a sensitive method for determining chlorine iodimetrically.

MAGNESIUM

The long-time standard procedure of determining this element in the filtrate from calcium, by precipitating with ammonium as the double phosphate to be weighed as magnesium pyrophosphate (**2**, p. 124), appears likely to remain the preferred method for some time. It is noteworthy that a colorimetric method is available for the determination of as little as 0.06 mg. of this element with an error of about 3 per cent. (**14, 16**). With the exception of use of the centrifuge to eliminate contamination by filter paper, the procedure is given fully in Chemical Abstracts **16**: 2342. 1922. Satisfactory use of a procedure involving precipitation of magnesium by 8-hydroxy quinoline is reported by the Department of Agricultural Chemistry, Purdue University.

PHOSPHORUS

The standard procedure for determining this element in fertilizer analysis is hardly applicable to the small amounts present in many types of plant tissue. Difficulty in filtering the ammonium phosphomolybdate, which may exist in too finely divided state as well as in extremely small proportions, is minimized by micro-procedure (**2**, pp. 130–131.). Essentially organic matter is destroyed by ignition with magnesium nitrate, and silicic acid is removed by the usual dehydration treatment. The phosphorus is then converted to ammonium phosphomolybdate, from which molybdenum may be reduced for accurate colorimetric measurement by comparison with a standard phosphate solution. It may be noted that the blue color of molybdenum here utilized has been applied to several determinations which permit the intermediate formation of a phosphate. Analysts in animal biochemistry have preferred aminonaphtholsulphonic acid over hydroquinone as the reducing agent, owing to off-colors of oxidation products and slower action in case of the latter. Nevertheless, hydroquinone appears to be satisfactory for multiple determinations in plant analysis.

POTASSIUM

The determination of this element gravimetrically by way of the chlorplatinate (**2**, pp. 125–130) seems to have been accorded a relatively permanent place in analytical procedures. In view of the considerable cost of the precipitant, it may be helpful to call attention to the possibility of determining potassium, either volumetrically or gravimetrically, as the cobaltinitrite. The volumetric phase of this procedure qualifies favorably as to both time and expense involved.

As formerly commonly conducted in the presence of acetic acid (**1**) this precipitation was attended by irregular results. VOLK and TRUOG (**31**) have investigated the requirements of this method thoroughly and have found

that the maintenance of carefully defined conditions during precipitation leads to reliable results. Chilling in an ice water bath during precipitation, and further also during filtration, serves to control the composition of the precipitate. Apparently one of the chief requirements of this method is that of holding cobalt in the oxidized state and thus permitting titrimetric oxidation of the nitrite radicle. SIDERIS (**27**) sought to avoid variability in composition of the cobaltinitrite precipitate by determining the cobalt colorimetrically. His procedure for thus determining potassium appears to add a slight element of complication. Attention should also be directed to the colorimetric procedure of MORRIS and GERDEL (**24**) based upon the cobalt content of the cobaltinitrite precipitate. Owing to the possibility of speed and replication, these investigators considered the method to compare very favorably with the chlorplatinate method. WILCOX (**33**) substituted nitric acid for acetic acid in the original cobaltinitrite procedure and thus held the cobalt in oxidized form at room temperature.

The following results were obtained by a moderately experienced analyst for a member of this committee, using twelve different samples of dried potato tuber tissue. The gravimetric determination of potassium as the chlorplatinate was used as a standard of reference for evaluating the volumetric cobaltinitrite procedure of WILCOX. Eight determinations by the volumetric method gave lower results than the gravimetric method, while four determinations gave higher results by the former procedure. The departure ranged from -7.4 to 1.4 per cent. of the corresponding gravimetric determinations, with an average departure of -2.3 per cent. It may be observed that the results are rather favorable to the relatively simple modification introduced by WILCOX.

SILICON

Although this element may occur in some samples primarily through contamination by soil, it is universal practice to remove it from the digest of the tissue ash. This precaution avoids contamination of some precipitates, and precludes interference with the phosphorus determination. Silicon is isolated by the familar procedure of evaporative dehydration of silicic acid contained in the acid digest of mineral matter. If samples of large silicon content are evaporated repeatedly, it may not be necessary to heat to 110°–120° as formerly specified. The presence of soil particles would necessitate a preparatory alkaline fusion. While the resultant silica may be weighed after filtration and ignition, it is more selective procedure to determine the loss of weight when it is volatilized as the silico-fluoride after treatment wth HF.

SODIUM

Apparently reliable, practicable methods for determining this metal are in the process of development. Until recently the A.O.A.C. has consistently

proposed that the contents of sodium be derived indirectly from the weight of mixed chlorides of sodium and potassium. This indirect procedure seems to have been rarely adopted, presumably because of infrequent interest in the determination of the element in question.

The A.O.A.C. (**2**, pp. 126–127) has presented a tentative gravimetric method for sodium, based on the formation of sodium-magnesium-uranyl acetate. BLENKINSOP (**5**) removed iron by gentle ignition of the ferric phosphate, added excess of titanous chloride, and titrated with iron alum. He found the procedure accurate to 0.1 mg. of Na. For those desiring greater refinement, it might be possible to adapt the photoelectric micro-procedure of HOFFMANN and OSGOOD (**19**). This method, based on stabilization of the yellow color of uranyl-zinc-sodium acetate against temperature changes by the addition of NH_4SCN, is reported as subject to an error of but 1 per cent.

SULPHUR

It is commonly known that the primary object in determining this element is conversion to SO_4 for precipitation as the very insoluble barium salt. The A.O.A.C. (**2**, p. 129) offers an option between fusing with a mixture of Na_2CO_3/Na_2O_2 and similar treatment with Mg $(NO_3)_2$, for the purpose of oxidation.

As in other determinations, the rôle of a proper blank determination on the reagents should be recognized. Should one not have access to the fundamental analytical procedures cited from the Methods of the A.O.A.C., these may generally be derived from standard treatises on quantitative analysis.

Determination of rarer mineral elements

As information accumulates, it appears possible that the list of elements which will eventually be included in this category may become relatively numerous. With the exception of iron, however, those which are now known to be deficient in soils to an extent that might be considered common, are but four in number. It was formerly supposed that spectrographic procedure provided the ultimate degree of accuracy for determining some of these elements. Doubtless the expense of apparatus for this physical method will prevent its use by many physiologists. Those who are in a position to secure assistance in this respect from departments of physics or chemistry may be considered fortunate. Nevertheless, as will appear presently, difficulties in adequacy of refinement inhere even in spectrographic equipment. Fortunately, the biochemist has recourse to colorimetric methods for determining several of these rarer elements. These procedures should be rather satisfactory for the purpose of comparison, which possibly expresses the requirement of the physiologist oftener than does absolute accuracy. There seems to be considerable applicability for colorimetric methods which may be accurate

to not over 95 per cent. It happens, however, that at least one such method is more sensitive than spectrographic procedure, excepting as the latter may be subject to refinement for evaluating the spectral lines. The difficulty of keeping up to date in this field is illustrated by current deficiencies in textbooks and manuals, as compared with the literature.

Boron

This element is subject to loss in ashing, primarily because of the formation of a volatile ester with glycerol from samples of high fat content. Preliminary impregnation of the tissue with a solution of either magnesium nitrate or sodium carbonate surmounts this difficulty.

Use has been made of the capacity of boric acid to esterify readily with methyl alcohol, for the purpose of isolation by distillation. Addition of mannitol to the free acid enhances the activity of the hydrogen ion so that it may be titrated. This type of procedure, as recommended by the A.O.A.C. for fertilizer samples, is inadequate for the low concentrations of boron encountered in plant tissues.

Preference has recently been indicated for a supposedly highly sensitive color reaction of boric acid with turmeric. This effect should be concentrated by capillarity at the top of a strip of indicator paper used wickwise (**29**, vol. 1, p. 526). The experience of an associate of a member of this committee with the turmeric method has been seriously disappointing, even with care as to factors controlling evaporation. Berger and Truog (**3**) have reported on the use of quinalizarin for the colorimetric determination of boron in soils and plants. This modification of a procedure developed by Smith (**28**) depends upon the color resulting from reaction with quinalizarin, and is reported to have the marked advantage of being applicable directly to digests of the ash.

From information now available, it appears advisable to separate the boron by esterification as directed in the Methods of the A.O.A.C. (p. 33) using, of course, boron-free glass. In addition, it will be necessary to use a photoelectric colorimeter in order to draw distinctions between amounts of boron in the vicinity of one part per million of the dry matter. For this purpose the use of a filter to transmit in the region of 6200 Å is advisable.

Attention may be called to a spectrometric procedure of Chalfee and McHargue (**7**). In this method the extinction coefficient of the spectrum of methyl borate is determined by means of a solution of potassium permanganate. The authors estimate that experienced machinists and glass technicians can assemble the apparatus at an expense of about $25.

Copper

The methods of the A.O.A.C. describe a colorimetric determination of this element in the form of its xanthate. A more sensitive procedure seems to be

available in the use of dithizone (phenylazothionoformic acid phenylhydrazide). This reagent reacts with a number of metals (see, for example, **18**). In presenting the dithizone method for copper, SNELL and SNELL (**29**, pp. 168–169) discuss some of its limitations. The requirements for preparation of reagents are somewhat exacting.

More reliable methods for copper involve the use of either pyridine thiocyanate (BIAZZO procedure, **4**) or diethyl dithiocarbamate (as used by CALLAN and HENDERSON, **6**). The former is the less complicated, while the latter is the more sensitive method. For application of the simpler procedure, see ELVEHJEM and HART (**11**), and for the other consult HADDOCK and EVERS (**15**).

IRON

The A.O.A.C. (**2**, p. 122) gives concise directions for the colorimetric determination of this element as ferric thiocyanate. SNELL and SNELL (**29**, pp. 283–298) discuss in detail the exacting requirement for avoidance of contamination, and the preparation of reagents. The latter recommend the addition of iron salt to the standard, and dilution as required, to duplicate the unknown. It is generally recognized that greatest reliability is secured by transferring both unknown and standard to a 1 : 1 mixture of amyl alcohol and ether for colorimetric comparison.

MANGANESE

It seems to be standard procedure to convert this metallic element to the form of potassium permanganate for determination. Apparently the procedure of the A.O.A.C. (**2**, p. 125) is fully acceptable.

ZINC

This element has been isolated commonly as the sulphide. SNELL and SNELL (**29**, p. 346) give directions for determining it nephelometrically (basis of turbidity) as the ferrocyanide.

When extreme accuracy is not essential, the dithizone (diphenylthiocarbazone) method of HIBBARD (**17**) may well be considered. This rapid procedure recovers 90 to 95 per cent. of zinc from amounts ranging from 0.001 to 0.100 mg. It entails scrupulous care for the use of zinc-free reagents and apparatus, to the extent of lubricating stopcocks by means of water. Interfering metals are eliminated by the application of solubility relations so that 3 to 30 gamma (thousandths mg.) of zinc are determined titrimetrically.

When extreme accuracy is desired, it will be well to consider the method of TODD and ELVEHJEM (**30**). Although their procedure seems to be involved, it is supposed to simplify and expedite the determination of this metal. Copper is separated from zinc by manipulation of the sulphides, and the latter metal is converted to its double phosphate with ammonium. A path is thus provided for determining zinc through its phosphorus equivalent.

The inorganic fraction of mineral elements

In numerous cases the significance of mineral determinations could be strengthened substantially if the relatively large proportion of the given element which existed in unassimilated form could be determined directly. Some time ago analysts gave attention to this issue. Perhaps phosphorus has been considered more frequently than other elements in this respect. Notable examples in this field are the papers of FORBES, BEEGLE, and WUSSOW (**12**), and CHAPIN and POWICK (**8**). As in other attempts to deal with the inorganic state, the problem appears to be that of inducing normal precipitation by overcoming the protective effects of colloids (especially of proteins). At the same time one must avoid the use of either reagents or conditional factors which might induce hydrolytic cleavage of phosphoric acid from organic compounds. FORBES and associates were unsuccessful in attempting to use either dilute watery or alcoholic solutions of mineral acids for extracting the inorganic phosphorus. On the other hand, CHAPIN and POWICK seem to have attained notable success in overcoming interference from proteins by extracting with dilute picric acid.

In recent years interest has developed in the determination of iron in the inorganic form. KOHLER, ELVEHJEM, and HART (**20**) have reported on the development of a procedure for determining available iron in foodstuffs by colorimetric use of the reaction with α-α'-bipyridine. They have encountered particular difficulty from the presence of tissue pigments. With fresh samples, interference from this source has been largely overcome by precipitation with lead acetate; but the difficulty has persisted with dried samples. An assistant to a member of this commitee was unable to apply the procedure satisfactorily to fresh potato tuber tissue.

Despite the paucity of methods, and the unsatisfactory nature of experience in this field, there appear to be elements of hope in the situation. If readers of these recommendations either have experience of value, or find it possible to extend the study of determining inorganic elements, it is hoped that they will make the experience available by publication. In this connection it may be well to consider the possible use of dialysis, and especially of electrodialysis under controlled voltages, as means of determining the degree of freedom of mineral elements. Examples of the application of these procedures are to be found in the literature.

THE UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. ADIE, R. H., and WOOD, T. B. A new method of estimating potassium. Jour. Chem. Soc. **77**: 1076–1080. 1900.

2. Assoc. Offic. Agr. Chem., Methods of analysis. 4th ed. Washington, D. C. 1935.
3. Berger, K. C., and Truog, E. Boron determination in soils and plants using quinalizarin reaction. Ind. & Eng. Chem. Anal. Ed. **11**: 540–545. 1939.
4. Biazzo, R. Rapid method for the determination of copper in preserved vegetable products which have been colored green artificially. Ann. Chim. Appl. **16**: 96–98. 1926.
5. Blenkinsop, A. A new volumetric method for the estimation of sodium. Jour. Agr. Sci. **20**: 511–516. 1930.
6. Callan, T., and Henderson, J. A. R. New reagent for the colorimetric determination of minute amounts of copper. Analyst **54**: 650–653. 1929.
7. Chalfee, R. K., and McHargue, J. S. Optical spectroscopic determination of boron. Polarizing attachments. Ind. & Eng. Chem. Anal. Ed. **9**: 288–290. 1937.
8. Chapin, Robert M., and Powick, Wilmer C. An improved method for the estimation of inorganic phosphoric acid in certain tissues and food products. Jour. Biol. Chem. **20**: 97–114. 1915.
9. Ellis, G. H. The microdetermination of calcium. Ind. & Eng. Chem. Anal. Ed. **10**: 112. 1938.
10. Elvehjem, C. A., and Lindow, C. W. The determination of copper in biological materials. Jour. Biol. Chem. **81**: 435–443. 1929.
11. ———, and Hart, E. B. A note on the Drabkin-Waggoner modification of the Biazzo method for determining copper. Jour. Biol. Chem. **91**: 37–42. 1931.
12. Forbes, E. B., Beegle, F. M., and Wussow, A. F. D. Studies on the estimation of inorganic phosphorus in plant and animal substances. Ohio Agr. Exp. Sta. Techn. Series Bull. 8. 1915.
13. Fraps, G. S. The determination of sulphur and chlorin in plants. North Carolina Agr. Exp. Sta. Ann. Rept. 25. pp. 44–49. 1901–1902.
14. Gadient, St. Über Mikrobestimmungen des Magnesiums. Helv. Chim. Acta **6**: 729–733. 1923.
15. Haddock, L. A., and Evers, N. Determination of minute amounts of copper in the presence of iron and certain other metals. Analyst **57**: 495–499. 1932.
16. Hammett, F. S., and Adams, E. T. A colorimetric method for the determination of small amounts of magnesium. Jour. Biol. Chem. **52**: 211–215. 1922; **54**: 565–566. 1922.
17. Hibbard, P. L. A dithizone method for measurement of small amounts of zinc. Ind. & Eng. Chem. Anal. Ed. **9**: 127–131. 1937.

18. ————————. Estimation of copper, zinc, and cobalt (with nickel) in soil extracts. Ind. & Eng. Chem. Anal. Ed. **10**: 615–618. 1938.

19. HOFFMAN, WILLIAM S., and OSGOOD, BESS. A photoelectric method for the microdetermination of sodium in serum and urine by the uranyl zinc acetate precipitation. Jour. Biol. Chem. **124**: 347–357. 1938.

20. KOHLER, G. O., ELVEHJEM, C. A., and HART, E. B. Modifications of the bipyridine method for available iron. Jour. Biol. Chem. **113**: 49–53. 1936.

21. LOOMIS, W. E., and SHULL, C. A. Methods in plant physiology. McGraw-Hill Book Co. 1937.

22. LUNDELL, G. E. F., and KNOWLES, H. B. Rapid detection of small amounts of aluminum in certain nonferrous materials. Ind. & Eng. Chem. **18**: 60–61. 1926.

23. McMURTREY, J. E., JR. Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. **4**: 183–203. 1938.

24. MORRIS, V. H., and GERDEL, R. W. Rapid colorimetric determination of potassium in plant tissues. Plant Physiol. **8**: 315–319. 1933.

25. PHILLIPS, T. G., SMITH, T. O., and DEARBORN, R. B. The effect of potassium deficiency on the composition of the tomato plant. New Hampshire Agr. Exp. Sta. Techn. Bull. 59. 1934.

26. SANDO, C. E. Lipides and their estimation in vegetable tissues. Plant Physiol. **3**: 155–184. 1928.

27. SIDERIS, C. P. Colorimetric microdetermination of cobalt and potassium. Ind. & Eng. Chem. Anal. Ed. **9**: 145–147. 1937.

28. SMITH, G. STANLEY. Determination of small amounts of boron by means of quinalizarin. Analyst **60**: 735–739. 1935.

29. SNELL, F. D., and SNELL, CORNELIA T. Colorimetric methods of analysis. 2nd ed. vol. 1, p. 526. 1936.

30. TODD, W. R., and ELVEHJEM, C. A. The determination of zinc in biological materials. Jour. Biol. Chem. **96**: 609–618. 1932.

31. VOLK, N. J., and TRUOG, E. A rapid chemical method for determining the readily available potash of soils. Jour. Amer. Soc. Agron. **26**: 537–546. 1934.

32. WILKENS, H. L. Report on total chlorine. Jour. Assoc. Offic. Agr. Chem. **21**: 353–354. 1938.

33. WILCOX, L. V. Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. Ind. & Eng. Chem. Anal. Ed. **9**: 136–138. 1937.

ABSORPTION AND MOVEMENT OF RADIOPHOSPHORUS IN BEAN SEEDLINGS

ORLIN BIDDULPH

Experimentation

Preliminary results using the radioactive isotope of phosphorus (P^{32}) as a tracer atom in Red Mexican beans (**2**) demonstrate the value of the method and its general application to plant physiological problems. Wherever it is desirable to follow the path or metabolism of single atoms, this procedure will prove extremely valuable.

The equipment used for the detection and measurement of the radiophosphorus consisted of a NEHER-HARPER high speed Geiger counter circuit (**14**) in connection with a direct-reading counting rate meter for random pulses as designed by GINGRICH, EVANS, and EDGERTON (**5**). A Geiger counter tube with a very thin glass window (**4**) proved satisfactory. The samples to be measured were held in cellophane cones which in turn were suspended in the concave sensitive area of the counter tube. Only a relatively small percentage of the total beta radiation emitted by the radiophosphorus entered the cathode cylinder of the counter tube; however, the geometric conditions remained constant for all determinations, and the counts per minute recorded represent a constant percentage of the total emanation. Results represent comparative amounts rather than absolute quantities.

The radiophosphorus was obtained through the courtesy of Dr. E. O. and Dr. E. H. LAWRENCE, of the Division of Radiation, University of California. It was made by bombarding 8-million volt deuterons against red phosphorus, which in turn was converted into sodium phosphate. The radiophosphorus-containing nutrient solution was made as follows:

Na_2HPO_4	0.0075 M	(contains the radiophosphorus)[1]
KCl	0.0020 M	
$Ca(NO_3)_2$	0.0020 M	
$MgSO_4$	0.0010 M	

The solution received continuous aeration. The conditions were not favorable for very rapid transpiration, as the light intensity fell from 2000 to 200 foot candles during the experiment. The temperature varied between 21° and 24° C., but was for the greater part of the experiment between 22° and 23° C.

Bean seeds, variety Red Mexican, were germinated and the plants grown in a complete nutrient solution until the fifth alternate leaf was unfolding.

[1] The final nutrient solution contained 0.76 μc/cc. of radiophosphorus.

They were then sorted for uniformity and three selected for tracer work. The first plant was placed in the radiophosphorus-containing nutrient solution for one hour, the second for two hours, and the third for four hours. After the required time, each was removed, the roots washed carefully and the plant dissected, dried in a 75° C. oven, ground to a fine powder and 10 mg. samples weighed for analysis. The remainder of the root tissues of each plant was extracted with ether in a Soxhlet, and then dried and extracted for three hours with successive portions of hot distilled water. The ether extract was evaporated, and determinations of radiophosphorus made on the residue. Aliquots of the water extract were also assayed. The results are shown in table I.

TABLE I

DISTRIBUTION OF RADIOPHOSPHORUS IN BEAN PLANTS GROWN IN NUTRIENT SOLUTION CONTAINING 0.76 μC/CC. RADIOPHOSPHORUS
(EXPRESSED IN COUNTS / MINUTE / MG. OF FRESH TISSUE)

Plant organs	Time in nutrient solution												
	1 hour			2 hours			4 hours			Combined roots			
	Tissue	Ether	Water	Tissue	Ether	Water	Tissue	Ether	Water	Water	Ether	2% HCl	Insol.
Roots	8.3	0	2.7	25.3	0.07	5.7	33.5	0.15	10.5	29%	1%	61%	9%
Hypocotyl and stem	0.0			1.1			0.6						
Opposite leaves	0.0			0.0			0.9						
1st alternate leaflets	0.0			0.0			0.5						
2nd " "	0.0			0.0			0.8						
3d " "	0.0			0.0			0.8						
4th " "	0.0			0.0			1.2						
5th " "	0.0			0.0			1.3						
Nutrient solution			11.8			11.0			10.4				

After finding that the water extraction removed only a comparatively small percentage of the total radiophosphorus, it was considered desirable to investigate the remainder. The tissue residues from the three roots were combined and extracted with 2 per cent. HCl. The phytin fraction is normally removed by this procedure and determined quantitatively by titration (1). The presence of ferric iron in the tissues, however, caused the precipitation of the insoluble iron-phytic acid complex, which remained in the tissue residue and consequently was not determined as such. The distribution of radiophosphorus in the combined roots can be calculated, and is as follows:

Water soluble	29%	2% HCl	61% (not phytin)
Ether soluble	1%	Insoluble	9%

The water soluble radiophosphorus in the tissues approaches that in the nutrient solution at the end of a 4-hour period.

Discussion

These results are of interest because the method employed enables the experimenter to follow absorption, movement, and accumulation of a nutrient salt in an intact plant.

Table I shows that the uptake of phosphorus under the conditions of the experiment was quite rapid. Radiophosphorus could be detected in the topmost leaves of the plant, a distance of approximately one meter from the base of the hypocotyl, within four hours after the roots were placed in the radiophosphorus-containing nutrient solution. This rate of uptake and movement is not as rapid as that reported by CRAFTS and BROYER (3) who detected Br in the xylem exudate of squash within 30 minutes after exposure of the roots to a solution containing 800 p.p.m. of KBr. With the bean plant, radiophosphorus was detected in the basal part of the stem within two hours after administration of radiophosphorus. It is very interesting to note the large amount of total radiophosphorus which was associated with the roots of the bean plants before appreciable quantities moved upward in the stem. It is not advisable to consider the total amount as being absorbed phosphorus, as relatively much may have been merely adsorbed on the surfaces. That which may be removed by hot water may represent more nearly the fraction which is normally free to move into the xylem vessels, and it can be seen from table I that the water soluble radiophosphorus associated with the roots approaches that in the nutrient solution after four hours of absorption. The relationship of the various fractions studied to the concentration of radiophosphorus in the nutrient solution can be seen from table I. It can also be seen that the time required for the movement of radiophosphorus into the stem corresponded fairly well with the time at which the total radiophosphorus associated with the roots reaches the same concentration as that in the nutrient solution. The proper interpretation of this point is questionable because of limited data.

The path of movement of radiophosphorus in the stem is unquestionably the xylem (15). It is impossible to account for the distribution throughout the plant assuming any other path. If movement were through the phloem (6) the concentration gradient in the aerial parts would necessarily be the opposite to that actually found. It appears, from the small amount of radiophosphorus in the hypocotyl (2) that on arrival of the radiophosphorus in the xylem tissue it is "swept" into the aerial parts by the transpiration stream. The distribution of radiophosphorus in the aerial parts corresponds very favorably to the transpiration rates of various leaves (13).

HEVESY, LINDERSTRØM-LANG and OLSEN (7, 8) present data from which

they contend that phosphorus migrates from leaf to leaf within the plant. They grew sunflower plants in a complete nutrient solution until a first set of leaves was formed, then transferred them to a nutrient solution containing radiophosphorus while a second set of leaves was formed. They then reason as follows:

". . . then we must distinguish between two extreme cases: (a) the phosphorus atoms do not migrate; (b) the phosphorus atoms migrate. In case (a) labeled phosphorus atoms should only be found in the upper leaves; in case (b) the labeled phosphorus atoms should be equally distributed between the upper and lower leaves . . ." (**8**).

Analysis showed that radiophosphorus moved into the lower leaves as well as the upper ones. This is, of course, no evidence for exchange of phosphorus atoms from leaf to leaf as the authors contend. It is merely evidence that phosphorus moved directly into the lower leaves as well as the upper ones through the vascular tissues supplying those leaves, and not that the radiophosphorus moved into the upper leaves only, and was then exchanged to the lower leaves. The same criticism applies to their work on maize (**8**). The above experiments were not properly designed to show migration of phosphorus from leaf to leaf. It is possible, however, for phosphorus to migrate from one leaf to another (**11**) chiefly upward, but it should be emphasized that the data of HEVESY *et al.* are not concerned with this type of exchange. A most interesting relationship between the work of MACGILLIVRAY (**11**), on the re-utilization of phosphorus by the tomato plant as the supply to the roots is removed, and the work of MASON and MASKELL (**12**) on the movement of phosphorus in the cotton plant, can be shown. The latter authors demonstrate that after the delivery of phosphorus to the leaves, via the xylem, there may be a downward movement through the phloem. They state that the downward movement in the phloem may be in excess of the amount required by the roots, and some of the mobile phosphorus may find its way back into the xylem and reascend the stem. This is the type of evidence which should be used to demonstrate migration of phosphorus atoms from leaf to leaf, and it is the only sense in which the term "migration" has meaning. No attempt seems to have been made to correct the erroneous impression of HEVESY *et al.*, as the inference is repeated in the new edition of "A Manual of Radioactivity" (**9**). It is hoped that, by calling attention to the above interpretation, a more correct appraisal of radiobiological activity may be made.

In tracing the movement of radiophosphorus in the bean plant, a concentration gradient has been found which corresponds to a transpiration differential. Accordingly, the transpiration stream delivers phosphorus to the leaves, where it accumulates as water is evaporated. This steepens the gradient in the phloem and allows a downward movement through that

tissue. If the supply of phosphorus to the root is suddenly removed, a diffusion of mobile phosphorus toward the xylem vessels would take place, and upon entry into the transpiration stream the ions would be "swept" upward again. This system of forces and gradients would account for the movement of ions which are water soluble, and which are known to accumulate in the upper parts of the plant as the supply to the roots is reduced or removed.

The very small amount of radiophosphorus in the ether soluble fraction within four hours after exposure to the nutrient solution is interesting, but previous work by WEBSTER and DALBOM (16) has shown that lipoid phosphorus constitutes only a small fraction of the total phosphorus of the mung bean. HEVESY and PANETH (9) state that radiophosphorus can enter into the phosphatide molecule only during its synthesis (in animals), and no evidence for the exchange of a newly acquired phosphorus atom for one already in chemical combination in a phosphatide molecule could be found. In the experimental work with the bean plant, it is highly improbable that ample time for much phospholipin synthesis was allowed, as the concentration of ether soluble radiophosphorus is still increasing at the end of four hours, the maximum period of contact with the nutrient solution.

Summary

1. The movement of radiophosphorus was traced throughout the bean plant. The total phosphorus associated with the roots was twice as great as the concentration in the nutrient solution before appreciable quantities entered the aerial parts.

2. The amount of water soluble phosphorus in the roots at the end of a four-hour period was equal to that in the nutrient solution.

3. Movement was rapid, and followed the transpiration stream.

4. Accumulation was greatest in the uppermost leaves.

5. An explanation of movement and accumulation is offered.

6. Criticism of the interpretations of HEVESY, LINDERSTRØM-LANG and OLSEN is offered.

The writer wishes to acknowledge with appreciation the assistance of the National Research Council in aiding this project, and to thank Dr. E. O. and Dr. E. H. LAWRENCE at the Radiation Laboratory of the University of California for the generous gift of radiophosphorus.

DEPARTMENT OF BOTANY
STATE COLLEGE OF WASHINGTON

LITERATURE CITED

1. ANDREWS, J. S., and BAILEY, C. H. Distribution of organic phosphorus in wheat. Ind. & Eng. Chem. **24**: 80–82. 1932.
2. BIDDULPH, O. Movement of radiophosphorus in bean seedlings. Science n.s. **89**: 393–394. 1939.
3. CRAFTS, A. S., and BROYER, T. C. Migration of salts and water into the xylem of the roots of higher plants. Amer. Jour. Bot. **25**: 529–535. 1938.
4. DUNNING, J. R., and SKINNER, S. M. Ionizing particle counters. R. S. I. **6**: 243–246. 1935.
5. GINGRICH, N. S., EVANS, R. D., and EDGERTON, H. E. A direct-reading counting rate meter for random pulses. Rev. Sci. Instr. **7**: 450–456. 1936.
6. GUSTAFSON, F. G., and DARKEN, MARJORIE. Upward transport of minerals through the phloem of stems. Science n.s. **85**: 482–483. 1937.
7. HEVESY, G., LINDERSTRØM-LANG, K., and OLSEN, C. Atomic dynamics of plant growth. Nature **137**: 66–67. 1936.
8. ———, ———, and ———. Exchange of phosphorus atoms in plants and seeds. Nature **139**: 149–150. 1937.
9. HEVESY, G., and PANETH, F. A. A manual of radioactivity. 2d Edition. pp. 174–175. Oxford Univ. Press. 1938.
10. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
11. MACGILLIVRAY, J. H. The importance of phosphorus in the production of seed and non-seed portions of a tomato fruit. Proc. Amer. Soc. Hort. Sci. **22**: 374–379. 1926.
12. MASON, T. G., and MASKELL, E. J. Further studies on transport in the cotton plant. Ann. Bot. **45**: 125–173. 1931.
13. MAXIMOV, N. A. The plant in relation to water. Transl. by YAPP. pp. 336–342. Allen and Unwin Ltd., London. 1929.
14. NEHER, H. V., and HARPER, W. W. A high speed Geiger-counter circuit. Phys. Rev. **49**: 940–943. 1936.
15. STOUT, P. R., and HOAGLAND, D. R. Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium, and phosphorus absorbed by roots. Amer. Jour. Bot. **26**: 320–324. 1939.
16. WEBSTER, J. E., and DALBOM, C. Changes in the phosphorus content of growing mung beans. Jour. Agr. Res. **41**: 819–824. 1930.

RESPONSES OF VARIOUS SPRING WHEATS TO VERNALIZATION

D. J. WORT

(WITH TWO FIGURES)

Introduction

There is little information in the literature regarding North American varieties of spring wheat which are responsive to vernalization treatment. The same variety has been reported by different investigators to react positively or negatively to treatment. For example, VAN HOEK (3) obtained acceleration of maturity when spring wheat was vernalized, but treatment of the same variety by GMELIN (1) gave no acceleration of flowering. WORT (4), using the same treatment with two different samples of Marquis wheat, found that the acceleration of flowering was 21 days in one case, and 2.6 days in the other, although growing conditions were similar. KOSTJUCENKO and ZARUBAILO (2) and others suggest from experiments with cereals grown in northern latitudes that at least part of the stage of vernalization (thermostage) may be completed during ripening and that this partial completion may reduce subsequent reaction to treatment. The environment, acting on a developing seed, affects the behavior of the plant produced by the seed.

Samples of various spring wheats (table I) obtained from the Departments of Agronomy at the State Universities of Illinois, North Dakota, and South Dakota,[1] were vernalized in an attempt to determine which varieties respond to vernalization and if varieties grown in different areas respond differently.

Methods

The procedure used for vernalization was as follows: (1) the amount of water, given in the formula used, was added to 20 gm. of seed in Petri dishes; (2) the seeds were allowed to germinate for 24 hours at 20° C.; (3) the Petri dishes were then stored for the required lengths of time in mechanical refrigerators at the temperatures given in the formulas. The lids of the Petri dishes were removed daily and the seeds aerated for a few seconds.

The factors in a formula in order are length of chilling in days, temperature during chilling in degrees C., and amount of water added in percentage of the original air-dry weight of the seed. Three formulas were used: (1) for early varieties, 6:10:50; (2) for medium varieties, 9:6:50; and (3) for late varieties, 12:4:50. These formulas are referred to by number in table I. Control seed was germinated 24 hours at 20° C., the water added being 50 per cent.

[1] The writer is indebted to Dr. G. H. DUNGAN of the University of Illinois; Dr. S. P. SWENSON of the University of South Dakota; and Dr. L. R. WALDRON of the University of North Dakota for the supplies of seeds used.

Five plants of treated or control grain were grown in 6-inch pots filled with well-mixed loam soil. Three replicates were used. All plantings were made on Sept. 9, 1939. Daylight was supplemented by 200-watt mazda lamps suspended 3 feet above the pots to give a 16-hr. day for the first 10 days, and an 18-hr. day thereafter. The plants were watered once a day. Flowering was assumed to have occurred when the anthers were first extruded.

Results and discussion

The results are recorded in table I. The source of the grain is stated in

TABLE I

VERNALIZATION TREATMENTS, AND ACCELERATION OF FLOWERING OF SPRING WHEATS

NAME OF VARIETY	SOURCE	EARLINESS RATING BY SOURCE	VERNALIZATION FORMULA	AVERAGE TIME TO FLOWER		ACCELERATION OF FLOWERING
				VERNALIZED	CONTROL	
				days	*days*	*days*
Bluestem	S.D.†	L	3	49.5	47.8	*−1.7
Caesium	S.D.	ML	2	46.1	48.0	1.9
Ceres	S.D.	ME	2	46.3	47.3	1.0
Comet	Ill.¶		2	48.7	52.4	3.7
General San Martin	Ill.		2	38.9	39.3	0.4
Haynes Bluestem	Chicago	L	3	49.0	49.1	0.1
Hope	S.D.	M	2	51.5	48.4	−3.1
Illinois 1 (Station)	Ill.		2	43.0	44.0	1.0
Illinois 1 (Mann)	Ill.		2	44.5	44.0	−0.5
Komar	Ill.		2	47.5	49.9	2.4
Marquis	S.D.	L	2	46.5	49.9	3.4
Marquis	N.D.‡	ML	2	49.1	47.6	−1.5
Marquis	Chicago	ML	2	47.1	48.9	1.8
Mercury	S.D.	ME	2	42.0	42.5	0.5
Merit	S.D.	ML	2	47.8	48.8	1.0
Ns2822	N.D.	VE	1	43.4	43.2	−0.2
Pilot	S.D.	ME	3	44.3	43.8	−0.5
Pilot	N.D.	L	3	48.4	49.7	1.3
Power	N.D.	L	3	44.8	46.8	2.0
Premier	S.D.	E	2	43.8	44.7	0.9
Premier	N.D.	ME	2	42.4	43.1	0.7
Progress	Ill.		1	43.0	43.1	0.1
Purdue	Ill.		2	42.2	45.3	3.1
Quality	S.D.	VE	1	37.9	38.3	0.4
Reliance × Prelude	S.D.	M	2	52.5	53.5	1.0
Renown	Ill.		1	41.8	42.6	0.8
Renown	N.D.	E	1	43.3	43.5	0.2
Reward	S.D.	VE	1	39.2	40.2	1.0
Rival	S.D.	ME	2	40.5	43.5	3.0
Rival	N.D.	ME	2	48.0	49.0	1.0
Sturgeon	Ill.		2	43.6	45.4	1.8
Thatcher	S.D.	E	1	45.8	45.4	−0.4
Thatcher	Ill.		1	48.6	47.8	−0.8
Thatcher	N.D.	E	1	46.8	48.5	1.7
Triumph	S.D.	VE	1	39.5	40.2	0.7
Vesta	S.D.	ME	2	41.3	40.7	−0.6

* Minus sign indicates a retardation in flowering

† S.D. = South Dakota.

¶ Ill. = Illinois.

‡ N.D. = North Dakota.

the second column and the rating of the wheat by local authorities is on the basis of very early (VE), early (E), medium early (ME), medium (M), medium late (ML), and late (L).

An examination of the table shows that a total of 27 samples responded to vernalization by acceleration of flowering and 9 were retarded by treatment. This ratio of 3 : 1 holds for the three main groups, early, medium, and late. Accelerations or retardations of less than one day, however, are probably insignificant. In some cases all samples of the same variety obtained from two or more sources (Marquis, Pilot, Premier, Renown, Rival, and Thatcher) responded similarly, and in other cases differently to treatment. Thus the accelerations of flowering of samples of Premier, obtained from North Dakota and South Dakota, were practically the same, being 0.7 and 0.9 days respectively. Marquis from South Dakota was accelerated in its flowering by 3.4 days; from North Dakota, retarded by 1.5 days; and a sample obtained in Chicago responded to treatment with an acceleration of 1.8 days. In a number of cases the relative flowering time of the samples grown in the greenhouse, did not correspond to the earliness rating given by the institutions supplying the grain.

The average height of the vernalized plants was slightly greater than

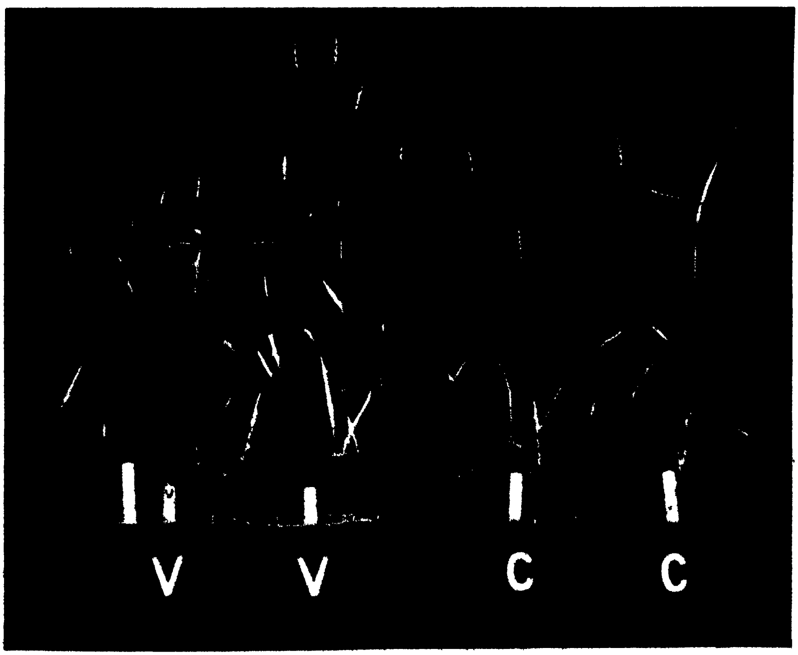


FIG. 1. Representative plants, South Dakota Marquis wheat, age 46 days. V = vernalized, C = control.

control in most cases; for example, Marquis (South Dakota) was 10.5 per cent. taller, and Thatcher (North Dakota) was 7.6 per cent. taller than their controls when measured on Oct. 31. The increased height was due to longer internodes. The appearance of these plants on Oct. 25 is shown in figures 1 and 2.

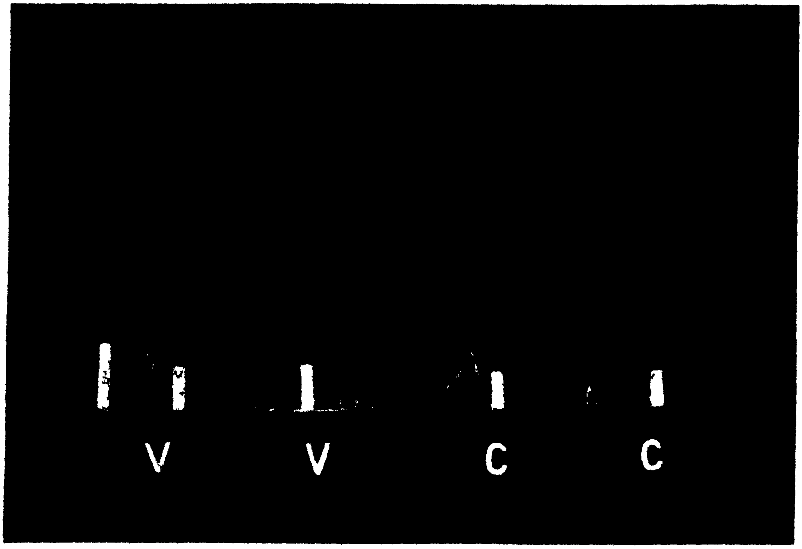


FIG. 2. Representative plants, North Dakota Thatcher wheat, age 46 days. V = vernalized, C = control.

The varied responses of the cereals make it very difficult to correlate the available weather data covering the period of flowering of the parent plant, and ripening of the seed later treated, with the seed's reaction to vernalization. It can be said, however, that the five varieties whose flowering was accelerated most by treatment—Comet (3.7 days), Marquis (3.4 days), Purdue (3.1 days), Rival (3.0 days), and Komar (2.3 days)—had been grown in South Dakota or Illinois where the temperature during flowering and ripening was higher than that in North Dakota. Of six varieties that had been grown in North Dakota and some other state only two of those produced in the more northerly area gave a greater response when tested.

While the vernalization formulas used may not have been optimum for all varieties, the results indicate those which are most responsive to treatment and hence most favorable for vernalization studies.

Summary

Thirty-six samples of various spring wheats from different regions in the United States were vernalized; of these, 27 responded with an acceleration,

and 9 with a retardation of flowering. Some varieties responded differently to treatment depending on the location where the seed had been produced, others responded similarly irrespective of their origin. The samples that responded most to vernalization were those that had been produced in areas with relatively high temperatures during the flowering and ripening of the parent plant.

DEPARTMENT OF BOTANY
THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. GMELIN, H. M. Report of department of crop cultivation of the University of Wageningen. Imp. Bur. Plant Genetics Bull. 17. 1935.
2. KOSTJUCENKO, I. A., and ZARUBAILO, T. J. Vernalization of seed during ripening and its significance in practise. Herb. Rev. **5**: 146–157. 1937.
3. VAN HOEK, S. Certain observations on vernalization. Landbouwk. Tijdschr. **46**: 809–814. 1934.
4. WORT, D. J. Vernalization of Marquis wheat and other spring cereals. Bot. Gaz. **101**: 457–481. 1939.

BRIEF PAPERS

EFFECT OF CHLOROPICRIN IN THE SOIL SOLUTION ON PLANT GROWTH[1]

FRANK L. STARK, JR., FRANK L. HOWARD, AND
JOHN B. SMITH

(WITH ONE FIGURE)

Previous experiments have shown that increased plant growth in chloropicrin-treated soil cannot be entirely explained on the basis of increased available nitrogen. Investigations have been carried on, therefore, to determine whether or not small traces of chloropicrin, which may remain in the soil following treatment, have any direct physiological stimulation upon plant growth. Several workers have concluded that volatile antiseptics used for soil treatment, when present in small quantities, have a stimulating effect on plant growth. KOCH (3) working with carbon disulphide and ether, concluded that residual traces of these substances affect the roots of plants, and he advanced the theory of direct plant stimulation. FRED (1) working with carbon disulphide and ether, holds that the increased growth of plants following the use of antiseptics in the soil depends essentially upon the stimulation of the plant itself. He finds (2), also, that in sand cultures supplied with soluble plant food, carbon disulphide favors the growth of certain plants. More recently, OLDENBUSCH (4) also concludes that small quantities of antiseptics exert a stimulating effect upon plant growth.

Because of the evidence cited the following experiment was set up to determine if it is possible that the beneficial effects of treating the soil with chloropicrin are of a direct stimulative nature. Ten drip culture pots were filled with washed, screened sand. Twenty-one buckwheat seeds were planted in each pot and grown by a drip culture method.

The culture solution contained the following concentrations of salts: calcium nitrate, 0.005 M; potassium dihydrogen phosphate, 0.001 M; potassium nitrate, 0.005 M; magnesium sulphate, 0.002 M; manganese sulphate, 0.00001 M; and boric acid, 0.0001 M. Dilute concentrations of chloropicrin were provided by adding varying amounts of a stock solution of chloropicrin to 180 liter volumes of culture solution. The flow of the solution was adjusted to a rate of about 6 drops per minute, approximately a liter per day.

The buckwheat plants were grown for thirty-five days and were then harvested. Because of the comparatively small root system developed by the buckwheat, the plants were cut off at the base of the stem and only the tops were weighed. The results obtained are shown in table I.

[1] Published by permission of the Director of Research as Contribution no. 557 of the Rhode Island Agricultural Experiment Station.

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF CHLOROPICRIN ON THE GROWTH OF BUCKWHEAT

POT	DILUTION OF CCl_3NO_2	NUMBER OF PLANTS	AVERAGE WEIGHT PER PLANT	WEIGHTED AVERAGE OF DUPLICATE TREATMENTS (GM. PER PLANT)
			gm.	*gm.*
1	No chloropicrin	20	5.78	5.89
2	1/500,000	16	4.00	4.10
3	1/1,000,000	20	4.45	4.47
4	1/2,000,000	20	5.45	5.09
5	1/3,000,000	20	5.38	5.11
6	No chloropicrin	18	6.00	
7	1/500,000	15	4.20	
8	1/1,000,000	19	4.50	
9	1/2,000,000	20	4.73	
10	1/3,000,000	20	4.85	

The plants came into flower at the same time regardless of treatment. All plants except those subjected to the highest concentration of chloropicrin were approximately the same height (fig. 1). The green weight was in-

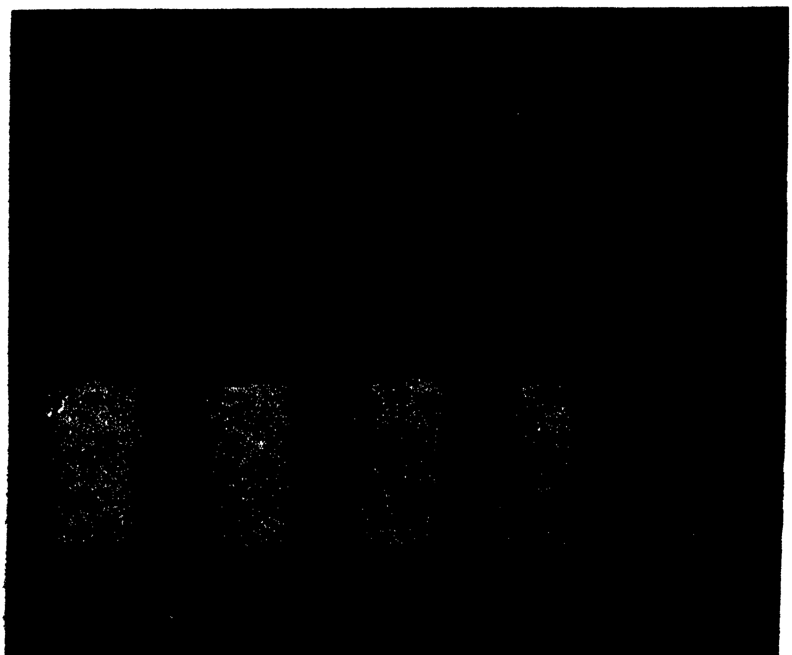


FIG. 1. Buckwheat plants grown by means of drip culture solution to which had been added various amounts of chloropicrin. Pots nos. 7 through 10 received decreasing amounts of chloropicrin, and pot 6 received no chloropicrin.

versely dependent upon the concentration of chloropicrin. In no case where chloropicrin was present, even in the most minute quantities, were the plants heavier than those developed in the plain culture solution. It is possible that concentrations lower than those used might stimulate plant growth. This is very improbable, however, because the lowest concentration was only one part of chloropicrin in three million of the culture solution.

In conclusion, the data appear to indicate that traces of chloropicrin in the soil solution are detrimental rather than stimulative to plant growth.

THE RHODE ISLAND AGRICULTURAL EXPERIMENT STATION
KINGSTON, RHODE ISLAND

LITERATURE CITED

1. FRED, E. B. Über die Beschleunigung der Lebenstätigheit höherer und niederer Pflanzen durch kleine Giftmengen. Centbl. Bakt. (II) **31**: 185–245. 1911.
2. ————. Relation of carbon disulphid to soil organisms and plant growth. Jour. Agr. Res. **6**: 1–19. 1916.
3. KOCH, A. Über die Wirkung von Äther und Schwefelkohlenstoff auf höhere und niedere Pflanzen. Centbl. Bakt. (II) **31**: 175–185. 1912.
4. OLDENBUSCH, C. Stimulation of plants by carbon disulphide. Bull. Torrey Bot. Club **49**: 375–390. 1922.

EFFECT OF NITRATE OF SODA ON THE RESPONSE OF SPINACH TO LENGTH OF DAY[1]

JAMES E. KNOTT

(WITH ONE FIGURE)

It is the common observation of growers that spinach plants which are closely crowded in the row, especially on mineral soil, will produce seedstalks more rapidly than where each plant has more room for development. On the peat soils of New York, spinach plants of the "slow bolting" varieties develop seedstalks very slowly under the long photoperiod of the summer months. It has been shown by WILSON and TOWNSEND (2) that the content of nitrate nitrogen in these soils may exceed 500 p.p.m. at a depth of 4 to 8 inches. This is equivalent to about 6.5 tons of nitrate of soda per acre foot. Considerably higher values are shown for the upper 4 inches of soil.

SCHAPPELLE (1), in studying the reproductive growth of Marchantia, radish, and spinach, found that cultures given distilled water without the addition of any nutrients were the first to respond to a photoperiod of 14 or 15 hours.

Iron drums 22 inches in diameter and 17 inches in depth were used as soil containers in this experiment. They were provided with a drainage hole in the bottom. Six drums were filled with a Dunkirk sandy loam soil of medium fertility and placed outside of the greenhouses where they were exposed to the prevailing weather and light conditions of the spring months at Ithaca. Seed of the Long Standing Bloomsdale variety of spinach was planted April 26, 1934. On May 7, the seedlings were thinned to a stand of 20 uniformly-sized plants in each drum. Nitrate of soda was applied to three of the drums beginning May 14. The rates of application were as follows: one ounce to each drum on May 14 and 21, June 11 and 18; four ounces to each drum on May 28 and June 5. The drums were watered well each time the nitrate was applied and an equal quantity of water was put on the check drums. The total application of nitrate of soda to each drum was equal to about 6 tons to the acre. The quantity of water applied, however, was such that it is likely that much of the nitrate was washed down to the lower levels of soil especially around the sides of the drums.

The spinach plants receiving nitrate were stockier, with foliage that was thicker, a darker green in color, and somewhat more savoyed than those which received no nitrate. Elongation of the central axis of some of the plants was first evident in both the treated and untreated plants on June 5. This was 40 days after planting. The photoperiod was then 15.1 hours. Al-

[1] Paper no. 192. Department of Vegetable Crops at Cornell University, Ithaca, New York.

though elongation commenced in some of the plants in all six drums at about the same time, most of the nitrated plants were slower in beginning seedstalk elongation than were those not nitrated. This is shown in table I by the percentage of seedstalks on June 14.

The heights of the seedstalks were measured on June 22. The means with their probable errors are given in table I. None of the tallest plants in the

TABLE I

PERCENTAGE OF PLANTS SHOWING ELONGATION OF THE CENTRAL AXIS ON JUNE 14, AND THE AVERAGE HEIGHT OF SEEDSTALKS ON JUNE 22 OF LONG STANDING BLOOMSDALE SPINACH GROWN WITH AND WITHOUT SIDE DRESSINGS OF NITRATE OF SODA

No nitrate added			Nitrated		
Drum	Plants showing seedstalks on June 14	Average seedstalk height on June 22	Drum	Plants showing seedstalks on June 14	Average seedstalk height on June 22
	%	mm.		%	mm.
A	80	241.8 ± 22.2	B	52	110.6 ± 17.7
C	77	230.3 ± 21.9	D	68	111.0 ± 16.2
E	88	157.1 ± 16.2	F	59	103.1 ± 14.2
Average of all plants		209.3 ± 12.4	Average of all plants		108.3 ± 8.9

FIG. 1. Foliage characteristics and relative seedstalk height of spinach plants photographed June 19. The plants in drum A received no nitrate while those in drum B were heavily nitrated.

nitrated drums was as tall as those not nitrated. This indicates that the seedstalks in the plants receiving nitrate elongated more slowly than did those to which no nitrate was applied. The type of growth on June 19 in drums A and B is shown in figure 1.

The large quantity of nitrate applied to this sandy loam soil has delayed seedstalk initiation and development. These results emphasize the importance of a consideration of the level of fertility of the soil in which are growing plants for use in photoperiodic experiments.

Department of Vegetable Crops
Cornell University
Ithaca, New York

LITERATURE CITED

1. Schapelle, N. A. Effect of narrow ranges of wavelengths of radiant energy, and other factors on the reproductive growth of long-day and short-day plants. Cornell University Agr. Exp. Sta. Memoir 185. 1936.
2. Wilson, B. D., and Townsend, G. R. Some nitrogen relationships in muck soils. Cornell University Agr. Exp. Sta. Memoir 137. 1931.

QUANTITATIVE STUDY OF THE PRODUCTION OF ETHYLENE BY RIPENING MCINTOSH APPLES

R. C. NELSON

(WITH ONE FIGURE)

Introduction

In an earlier paper, (4), the author has described the relationship which exists between ethylene production and keeping quality in several varieties of apples, and noted the similarity in shape of the curves for respiration and ethylene production. In the present paper ethylene production has been studied in relation to respiration and the ripening process.

Material and methods

The method used was similar to that which had already been used by the author (5) in studies of the banana. The fruit was placed on a wire stand beneath a bell-jar together with a known amount of base to absorb the carbon dioxide formed in respiration. The carbon dioxide absorbed was replaced by oxygen under low constant pressure from a gasometer, thus maintaining a normal atmosphere in the jar while permitting the accumulation of emanations from the fruit.

After the fruit had been under the bell-jar about 20 hours, a sample of the gas in the jar was taken for analysis. The fruit was then aired in preparation for the next day's run. The amount of carbon dioxide produced was determined by diluting the base to a suitable volume, adding barium chloride to an aliquot to precipitate carbonate, and titrating the free base to phenolphthalein with standard hydrochloric acid. All results were reduced to a 24-hour basis and are so expressed.

In the present experiment McIntosh apples obtained locally were used, 4.30 kg. of fruit being placed under the bell-jar. The experiment was carried out in a room maintained at 20° C.

Results

The results obtained are shown graphically in figure 1. At the beginning of the experiment the apples, judged by taste, respiratory activity, and the presence of starch, were unripe. The digestion of starch began on the fourth day, when the respiration was at its maximum (the climacteric) and was practically complete on the seventh day. The progress of starch digestion was observed by use of the iodine test on sections of tissue. The characteristic odor of ripe McIntosh apples was first present on the fifth day.

Respiratory activity increased rapidly up to the fourth day, after which it declined slowly. KIDD and WEST (3) have shown that this respiratory maximum at the time of ripening is characteristic of apples.

The rate of emanation of ethylene remained low during the first 3 days and then increased rapidly to the ninth day, after which it began to decline. The maximum of ethylene production occurred five days after the respiratory maximum.

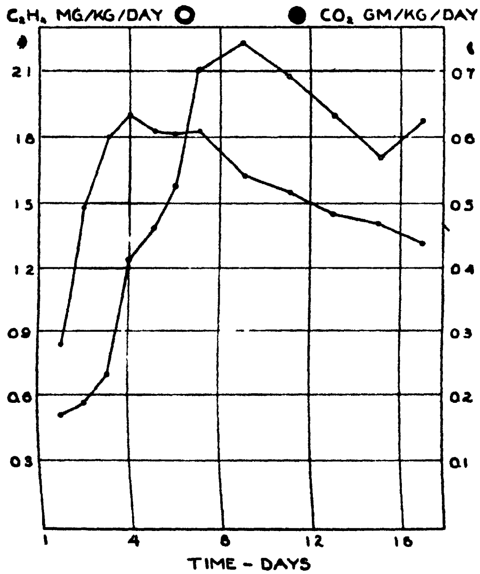


FIG. 1. Respiration and ethylene production of McIntosh apples during ripening.

Discussion

It will be observed that there is an inflection in the curve (fig. 1) of ethylene emanation on the fifth and sixth days corresponding with the period of active ripening. This inflection is probably attributable to the consumption of ethylene in the ripening process, such as the author has demonstrated in bananas (5).

The effects of ethylene on plant respiration are well known. DENNY (1) found that ethylene in concentration of 100 p.p.m. in air stimulated the respiration of lemons. HARVEY (2) has found that ethylene in concentration of 2 p.p.m. at first stimulated and later inhibited respiration of pea seedlings. One would predict, accordingly, that some relation would exist between ethylene production and respiration in the apple. No simple relationship appears in the present data. It is possible, however, that the low preclimacteric concentrations of ethylene are stimulatory, causing the onset of the climacteric, and that the higher concentrations are depressive causing the decline in respiration after the climacteric.

It seems likely that ethylene does play some essential part in the ripening process in both apple and banana, but it is not yet possible to say exactly what its rôle can be.

Conclusions

The rate of ethylene production in the McIntosh apple begins to increase rapidly after the onset of the climacteric, and reaches a maximum several days later than the respiratory maximum. Evidence is presented to show that, as in the banana, ethylene is consumed during the ripening process in McIntosh apple.

THE UNIVERSITY OF MINNESOTA
ST. PAUL, MINNESOTA

LITERATURE CITED

1. DENNY, F. E. Effect of ethylene upon respiration of lemons. Bot. Gaz. **77**: 322–329. 1924.
2. HARVEY, E. M. Some effects of ethylene on the metabolism of plants. Bot. Gaz. **60**: 193–214. 1915.
3. KIDD, F. and WEST, C. The course of respiratory activity throughout the life of the apple. Rept. Food Invest. Board 27. 1924.
4. NELSON, R. C. Studies on the production of ethylene in the ripening process in apple and banana. Food Research **4**: 173–190. 1939.
5. ————. The production and consumption of ethylene by ethylene treated bananas. Plant Physiol. **14**: 817–822. 1939.

FLAX VARIETIES COMPARED

J. C. Ireland

The introduction of a crop involves the question of quality as well as the environmental conditions under which profitable production may be undertaken. Varieties enter into the quality of the crop. In order to solve this problem in the case of flax, a comparative study of the available samples of seed was made, following the routine methods for "oil seeds." Samples were obtained from four states besides Oklahoma where flax is grown for the seed. Experienced seedsmen or experiment station workers labeled the varieties, and we assume that they are true to name. The object of our work has been to determine which variety is most desirable from the standpoint of oil and protein production and to determine if there is a climatic limitation for flax seed production.

The Wintons (1) quote Haselhoff, showing that flax obtained from widely separated areas has a fairly uniform composition. The differences might have been due to varieties or soil conditions.

Methods

The Official Methods of the A.O.A C. were used in determining the mois-

TABLE I

Comparison of flax varieties from five states

Variety	Water	Protein	Oil	Iodine number	Ash	Weight 1000 seeds
	%	%	%		%	gm.
Kansas						
Bison	10.43	38.10	34.49	194.6	3.96	6.13
Linota	9.57	31.19	27.78	190.6	4.15	3.94
Redwing	9.02	33.87	24.02	199.3	3.14	4.22
Viking	8.67	35.37	33.82	200.0	3.00	6.18
Minnesota						
Bison	7.24	40.81	34.44	201.5	3.27	5.85
Redwing	10.15	29.44	24.96	177.4	3.08	4.28
North Dakota						
Bison	9.43	41.50	32.66	181.8	4.05	5.70
Buda	9.52	42.62	34.33	186.6	3.29	4.27
Linota	9.10	37.12	27.71	194.0	3.26	3.78
Oklahoma						
Redwing	8.71	31.56	25.89	170.3	3.53	4.29
Linota	9.13	33.54	28.20	179.4	2.82	3.81
South Dakota						
Bison	9.75	40.09	31.07	179.3	3.91	4.20
Buda	10.10	40.25	34.05	188.9	3.58	4.31
Linota	9.50	37.88	31.87	200.7	3.39	3.95
Redwing	8.78	36.50	29.39	195.8	3.53	4.72

TABLE II

SUMMARY OF FLAX VARIETIES

VARIETY	WATER	PROTEIN	OIL	IODINE NUMBER	ASH	WEIGHT 1000 SEEDS
	%	%	%		%	gm.
Bison	9.21	40.12	33.16	189.3	3.79	5.37
Buda	9.81	41.43	34.19	187.7	3.43	4.14
Linota	9.32	34.93	28.89	191.2	3.41	3.87
Redwing	9.16	32.84	26.06	185.7	3.32	4.38
Viking	8.67	35.37	33.82	200.0	3.00	6.18

TABLE III

SUMMARY OF FLAX SEED BY STATES

STATE	WATER	PROTEIN	OIL	IODINE NUMBER	ASH	WEIGHT 1000 SEEDS
	%	%	%		%	gm.
Kansas	9.18	34.63	30.03	196.1	3.56	5.11
Minnesota	8.69	35.14	29.70	189.4	3.17	5.02
North Dakota	9.35	40.31	31.56	187.8	3.53	4.58
Oklahoma	8.91	32.55	27.04	174.4	3.17	4.05
South Dakota	9.53	38.68	31.59	191.2	3.60	4.24

ture and protein content. For this determination, the seed was ground with a food chopper. The oil was extracted in Soxhlet extractors by petrol ether. The residue in the thimbles was used for the protein determinations. The Hanus iodine number was determined according to the Official Methods. The weights of 1,000 seeds are importont in showing the principal varietal difference in flax.

Results

Table I summarizes the data. Unfortunately, it was difficult to obtain samples of all varieties from all the states. Inspecting the results for the variety Bison, it may be observed that it is comparatively high in everything, wherever grown. Seed catologues describe it as a product of the North Dakota Experiment Station, resistant to rust and wilt. Buda is perhaps equal to Bison, but it seems to be available only in the Dakotas. The more widely distributed Linota and Redwing do not have the oil and protein values of the others. The Viking flax from Kansas is a golden-seeded variety that may become a very popular type.

Table II averages all the data from table I under the respective varieties. State summaries are included in table III. The apparent superiority of the seed from the Dakotas may be attributed to the fact that it was especially selected while those from other states were more nearly "field runs." Flax

variations appear to follow varietal differences, rather than the environmental factors found in different states.

Bison and Buda seem to be somewhat superior to other varieties, not only in composition but probably in disease resistance.

OKLAHOMA AGRICULTURAL AND MECHANICAL COLLEGE
STILLWATER, OKLAHOMA

LITERATURE CITED

1. WINTON, A. L., and WINTON, KATE B. The structure and composition of foods. John Wiley and Sons. New York. 1932. (Vol. 1, pp. 529–535).

NOTES

Annual Meeting.—The sixteenth annual meeting of the American Society of Plant Physiologists was held at Columbus, Ohio, December 28–30, 1939. The meeting was well arranged, and excellent programs were presented. For the efficient and painstaking service of the program committee, special notice and thanks are due to Dr. B. S. MEYER of Ohio State University, chairman of the committee, and to his assistants, Dr. J. D. SAYRE and Dr. R. O. FREELAND. Everything that could be done to insure a comfortable and profitable meeting was done with dispatch. The officers, also, President JOHN W. SHIVE, and the Secretary, Dr. W. E LOOMIS, gave freely of their time and energy to insure a successful meeting.

The meetings were featured by several interesting and valuable symposia. One of these, on December 29, participated in by horticulturists and plant physiologists, dealt with the *Physiological Processes of Plants in Relation to Temperature.* The papers were well prepared, and presented a remarkable array of facts regarding the influence of temperature upon the absorption of water and solutes, photosynthesis, growth, flowering, and fruiting behavior of plants. A very long symposium on *Photosynthesis* was held under the auspices of Section C, A.A.A.S. The papers presented in this symposium should be collected and published, so that all workers might be able to read and study them more carefully. A symposium on the teaching of plant physiology occupied a late hour on Friday evening, December 29, under the leadership of Dr. O. F. CURTIS, of Cornell University. It attracted a deeply interested group whose problems with the teaching of plant physiology are many and varied. These teaching discussions are helpful, and deserve encouragement. Each one carries away some practical ideas of improvement of technique which must in some measure lift the general level of our teaching programs.

The annual dinner, held in the Junior Ball Room of the Neil House, Columbus, drew a large group together. Approximately 200 members and friends enjoyed the fellowship of the evening. Dr. WALTER F. LOEHWING, retiring president, read a very interesting paper entitled *The Effect of Mineral Nutrients on Flower Physiology,* and President SHIVE called upon the life membership committee, Dr. EARL S. JOHNSTON of the Smithsonian Institution, chairman, to report the selection of a recipient of the sixteenth award of the CHARLES REID BARNES life membership. The report was received with great interest and applause. The committee deserves the highest commendation for its excellent service. The report of the award is made in the following note.

Taking everything into consideration, the sixteenth annual meeting was

very well organized, very successful, and reflects the fine service of the officers and committees responsible for its success.

Life Membership.—The sixteenth award of the CHARLES REID BARNES life membership was made to Dr. W. J. V. OSTERHOUT, who, owing to an unfortunate illness, was unable to be present to receive the award. Dr. OSTERHOUT, who has been connected with the Rockefeller Institute for Medical Research for many years, is the author of many papers dealing with the problems of permeability, antagonism, and bioelectric potentials in living cells. These contributions have won him world-wide recognition, and he has given a life-time of service in the field of plant physiology. His was among the first one hundred names to be starred in *American Men of Science* in 1906. Born at Brooklyn, New York, August 2, 1871, he was educated at Brown University, where he won his A.B. degree in 1893, and A.M. in 1894. After a period at Bonn in 1895–1896, he returned to the United States, and received his Ph.D. from the University of California in 1899. He was instructor at Brown University for two years, 1893–1895, and rose through the ranks from instructor to associate professor at the University of California from 1896 to 1909. In this latter year he was called to Harvard, where he served for 16 years. In 1925 he became a member of the Rockefeller Institute for Medical Research. He was a joint-editor of the Journal of General Physiology, and a member of the Board of Trustees of the Marine Biological Laboratory, Woods Hole, Massachusetts. He served also for some years on the Board of Directors of the Rockefeller Institute. He has been the recipient of many honors, degrees from Harvard (1925), and Brown (1926); membership in the National Academy of Sciences; and honorary membership in numerous foreign societies.

In making its selection, the committee has made a very happy choice, one by which the Society is deeply honored. We are grateful that Dr. OSTERHOUT has been added to the roster of CHARLES REID BARNES life members.

Southeastern Section.—In accordance with provisions of the constitution, a petition duly signed by a large group of members residing in the southeastern states came before the American Society of Plant Physiologists at Columbus, asking that they be granted a charter for the establishment of a Southeastern Section of the Society. The petition set forth the territory to be included, as follows: North Carolina, South Carolina, Florida, Tennessee, Mississippi, Louisiana, Alabama, Georgia, and the West Indies. The temporary officers are Dr. G. M. ARMSTRONG, of Clemson College, chairman, and Dr. T. J. HARROLD, University of Georgia, secretary. The petition was favorably received by the executive committee, and on presentation before the business meeting of the Society, was given a unanimous vote of approval.

The first meeting of the new Southeastern Section will be held in the near future, and permanent organization will be effected. We hope that the development of this section will prove beneficial to the region it serves. This is the third regional section, and the fifth section to be established. We extend congratulations to the officers and members upon the successful outcome of their efforts. They will automatically obtain representation upon the executive committee of the Society, through the election of a permanent chairman, who then becomes a member of the executive group. We wish the new section every success with the organization.

Program Committee.—The program committee for the seventeenth annual meeting at Philadelphia in 1940 will be headed by Dr. WILLIAM SEIFRIZ of the University of Pennsylvania. The success of a meeting depends very much upon the convenience of arrangements, and it is a great advantage to have the chairman of the program committee on the grounds, and thoroughly familiar with all of the problems. Philadelphia was the scene of the third annual meeting of the American Society of Plant Physiologists. During the fourteen-year interim great progress has been made; it should be fully reflected in the program and the attendance. Cooperation of all members is desired in making this meeting our most important one to date.

Manuscripts.—The editor-in-chief desires to repeat the request that all manuscripts be sent originally to the office of the secretary of the editorial board, Dr. WALTER F. LOEHWING, Department of Botany, State University of Iowa, Iowa City, Iowa. Serious delays will be encountered by papers sent directly to Chicago.

The method of handling papers was slightly modified at the Columbus meeting, to preserve the benefits of having the papers examined by competent readers, but to allow expedition in reaching a decision on the papers submitted directly to Dr. LOEHWING.

In view of the critical financial situation of many of our subscribers, we are requesting authors to put their work into as concise form as possible, with few tables and cuts, and few mathematical formulae. These are the most expensive items in the printer's statements. Text is relatively inexpensive, but may be shortened by concise treatment of methods and previous literature.

Drawings should be made not larger than 8.5×11 inches, which require reduction to one-half or one-third to fit the pages of PLANT PHYSIOLOGY. We can no longer accept rolls of enormous-sized drawings. Authors submitting larger drawings than regular size will be requested to send other copies of the right size, or photographic reductions, before they are submitted to the readers.

Reprint Orders.—It is desirable to have all reprint orders filed in the editorial office at Chicago. When orders are sent directly to Lancaster, the editor-in-chief cannot give the orders the attention that they should receive. There is no objection to the making out of official orders to the Science Press Printing Co., but these orders should be filed with the editor so that they may be transmitted to the publishers with the page proofs. The green order blanks should represent the entire number of reprints desired by the author and his institution. If the shipment is to be divided between the author and the institution, directions should be given for the two shipments on the blanks designed for this purpose, at the lower end of the reprint order blank. Errors are difficult to avoid unless the author cooperates with the editor-in-chief by sending full and complete information on the order, and the official papers issued by his institution.

Endowment Funds.—A slight change was made in the endowment fund accounting at the sixteenth annual meeting. The life membership endowments are to be consolidated with the general endowment to simplify the book-keeping. There has been a healthy growth of the general endowment during the last year or two. Those who are able to do so may aid in the building of these funds by purchasing journals that are over five years old. Gifts are always welcome, and the finance committee will be very glad to receive funds for investment at any time. A general endowment of $25,000 would enable us to publish all papers promptly. It would also enable us to use an occasional colored plate when color is necessary to an understanding of the results. Thus far, all colored plates used in PLANT PHYSIOLOGY have been presented by institutions interested in the appearance of the papers with colored plates. At the close of the fiscal year, June 30, 1940, a statement showing the condition of the endowment funds will be published, in the hope that many may be encouraged to help increase them.

Robert Marshall.—On November 11, 1939, death came to Dr. ROBERT MARSHALL, Chief of the Division of Recreation and Lands of the U. S. Forest Service. He had not quite completed his 39th year. He was born in New York on January 2, 1901. His degrees were obtained at Syracuse (B.S., '24), Harvard (M.F., '25), and Johns Hopkins (Ph.D., '30). For several years after completion of his forestry studies at Harvard, he was a member of the staff of the Northern Rocky Mountain Forest Experiment Station (1925–1928). He was engaged in research for four years, until 1932, when he again was connected with the Forest Service. He spent one year as collaborator, and then was made director of forestry and grazing in the Bureau of Indian Affairs in the Department of the Interior, a position which he held for four years. In 1937 he became Chief of the Division of Recreation and

Lands, the position he held at the time of his death. During the period of his research, he was a member of an expedition to the arctic regions of Alaska for the study of tree growth and civilization.

Dr. MARSHALL was a life member of the American Society of Plant Physiologists, was also a member of many scientific organizations, and a member of the Explorers' Club. It is unfortunate, indeed, that one so well prepared for his work, one who had made such rapid progress in his chosen field, should so early be taken from us. He is deeply missed and mourned by his many colleagues and friends.

Quantitative Biological Spectroscopy.—During recent years great use has been made of spectroscopy in the quantitative aspects of biological investigation. This is especially true in the quantitative estimation of pigments, and in the identification and purification of such substances. Dr. ELMER S. MILLER, of the University of Minnesota, has published a very valuable monograph on the applications of spectroscopy to biological problems under the title *Quantitative Biological Spectroscopy.* The publishers are the Burgess Publishing Co., Minneapolis, Minnesota. There are 18 chapters, covering concisely, but still thoroughly, the necessary material. It begins with a short history of optics, and an introduction to molecular spectra, then takes up the instruments, such as thermopiles, photoelectric cells, amplifiers, and spectrophotometric instruments. These chapters are followed by the methods of spectrophotometric and photoelectric photometry, the basis of absorption spectra study, sources of error, and the application of the methods to biological chemistry. Some of the later chapters consider the absorption spectra of vitamines, cytochromes, hemoglobin derivatives, lipids, and the analysis of binary, ternary, and quaternary mixtures. The work closes with a bibliography of 163 titles, annotated. The reviewer believes that many workers will find this book a mine of valuable information; for, whether we work in the field or not, we need the information to understand the work being done by spectroscopists. The contributions in this field have been extremely valuable, so the general worker should understand the nature and validity of the results obtained.

The book is mimeoprinted, with many illustrations, and occupies 213 pages. The price, $3.50, is very reasonable for a work of this type. Orders may be sent to Burgess Publishing Co., 426 South Sixth St., Minneapolis, Minnesota.

Elements of Plant Physiology.—It is with a great deal of pleasure that we announce the appearance of *Die Elemente der Pflanzenphysiologie,* by Dr. P. BOYSEN JENSEN, Professor of Plant Physiology at the University of Kopenhagen. This work first appeared as *Plantefysiologi* in the Danish

language. It has now been translated into German by Dr. FRITZ MATTICK of the Botanical Museum, Berlin-Dahlem. The work is well organized. The introductory chapter distinguishes lifeless and living material, and considers the influence of external and internal factors upon living organisms, and the stimulus responses of living organisms. The second chapter deals with the gross structure, and anatomy of plants, the chemical constitution of plant substances, and the submicroscopic structures, with accompanying surface phenomena. This chapter closes with a consideration of the behavior of plants toward extreme conditions of temperature, light, drought, hydrogen-ion concentration, and poisons.

Then follow chapters on material intake, material outgo, and translocation of material through the plant body; energy release and energy transformations; assimilation; growth and development, and orientation movements of plants and their organs. Special attention is called to the treatment of growth and responses. These chapters should be familiar to all teachers of plant physiology, as they represent the views of one of the world's greatest students of hormones in relation to plant responses.

The author's style is direct, the translation has been skilfully accomplished, and readers will not experience difficulty in reading it, if they are moderately proficient with German. We commend it to all students of plant physiology as a text with which they should be familiar. It is well illustrated (162 text figures), and is provided with a good index. The publisher of this excellent work is Gustav Fischer, Jena, who quotes it at RM 24 per copy in paper binding.

Magic Gardens.—Lovers of old-fashioned gardens will find much pleasure in reading *Magic Gardens,* by ROSETTA E. CLARKSON. It is a Macmillan Co. publication, and a fine addition to their long list of garden books. This work deals with fragrant herbs and savory-seeded plants, used more in bygone ages than now. The book is like a breath of fragrance out of an Elizabethan garden, and the illusion is maintained by numerous illustrations taken from ancient herbals and old books on gardening. One can find in it inspiration to have one's own garden of old-fashioned herbs, of anise, basil, burnet, caraway, sweet marjoram, rosemary, and thyme. Moreover, it has the value of an historical document, with its illustrations from such works as PARKINSON'S *Paradisus in Sole* (1629), EVELYN'S *French Gardiner* (1675), GERARDE'S *Herball* (1597), LIGER'S *Le Jardinier Fleuriste* (1763), LAWSON'S *A New Orchard and Garden* (1618), and many other early sources of information. It is recommended especially to those who practice gardening for the love of it, to those in whose memories linger the odors of freshly turned soil, of spring flowers, of luxuriant woods vegetation, of grandmother's sage and hoarhound. The cost of this book is only $3.00, and its possessors will receive large dividends in pleasure.

FRANK MARION ANDREWS
JULY 27, 1940

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

FRANK MARION ANDREWS

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 27, 1870

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

FRANK MARION ANDREWS

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 27, 1870

FRANK MARION ANDREWS
JULY 27, 1870

Vol. 15 No. 2

PLANT PHYSIOLOGY

APRIL, 1940

FACTORS AFFECTING REGENERATION OF THE HORSERADISH ROOT[1]

ROBERT C. LINDNER

(WITH TWELVE FIGURES)

Introduction

The term "regeneration" is ordinarily used to indicate the formation anew of a part of an organism that has been lost. In this paper the term is applied to the initiation of buds and roots from isolated pieces of the horseradish root. The growth of any plant axis, whether it be root or stem, may be separated into at least two phases: (1) The actual initiation of a growing point from a mass of meristematic tissue, and (2) the maintenance and subsequent development of this growing point. In the work reported here, no attempt was made to analyze the factors involved in the subsequent development of buds and roots after they were initiated. Reviews of the older literature on regeneration may be found elsewhere (**9, 12, 15, 16**).

The horseradish, *Cochlearia armoracia,* is a convenient plant for a study of this type, because small isolated pieces of the root regenerate both buds and roots quite readily when placed in the proper environment. Furthermore, these pieces are fairly resistant to invasion by microörganisms. Previous work (**13**) has shown that normally the initiation of buds and roots is confined to those regions of the root where a lateral root has been broken off. Both buds and roots apparently arise from identically the same region. It has also been shown that the application of relatively high concentrations of indoleacetic and naphthyleneacetic acids inhibits the development of buds and stimulates the development of roots. The purpose of this paper is to elucidate some of the factors involved in the initiation of buds and roots, with particular emphasis on bud initiation.

Methods

Horseradish roots were obtained from a market in Chicago and stored at 2°–4° C. until used. If the humidity of the storage room is kept high

[1] Contributions from the Hull Botanical Laboratory no. 513.

enough to prevent excessive desiccation, the roots may be stored for months at this temperature without apparent detrimental results. Roots of approximately the same diameter (20–28 mm.) were used to insure more uniform test material. When a root was prepared for use, the upper and lower portions were discarded, leaving the central portion for experimental purposes (fig. 1). Adhering dirt was removed by scrubbing with a stiff-bristled brush

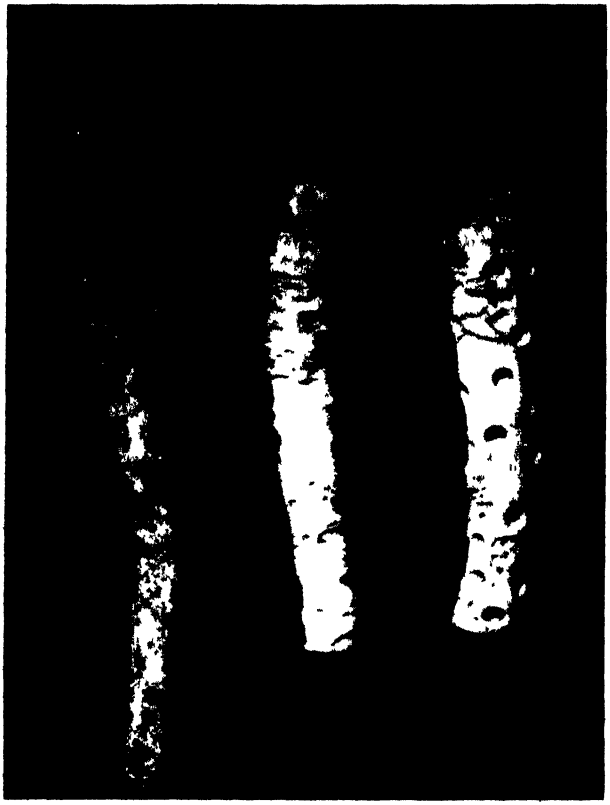


FIG. 1. Stages in preparation of horseradish root: (1) dormant root; (2) after trimming and washing; (3) after removal of test pieces. The ends of the root traces are visible as small elevations on the surface of the root.

while washing thoroughly with water. Care was taken to prevent injury to the root during this process. With a short cork-borer, a small piece of root was removed containing a root trace and its surrounding tissue. The piece was removed from the cork-borer by pressing on the end opposite the periderm with a glass rod (fig. 2A). A short cork-borer is advantageous in this procedure; the one used in most of the work was 6 mm. in diameter and 30

mm. long. After removal from the cork-borer, a test piece of the desired length (usually either 1 mm or 3 mm.) was cut with a sharp razor, and the remaining tissue discarded. Ten such test pieces were placed with cut ends down on clean moist sand in a Petri dish (fig. 2B) and kept in the dark at

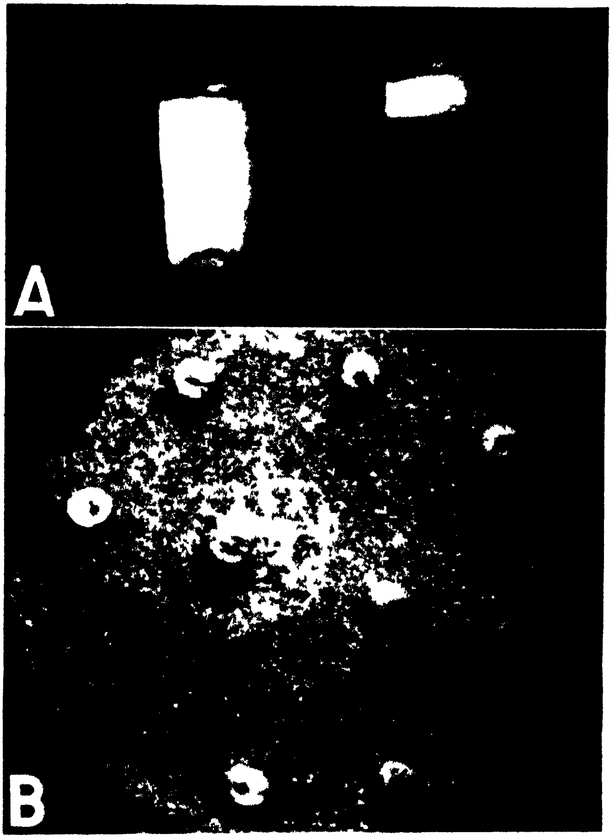


FIG. 2. Preparation of test pieces. A: piece on left as removed from cork-borer; piece on right trimmed to 3 mm. test piece (×2). B: test pieces on moist sand in Petri dish. The ends of the root traces are visible as small elevations on the surface of the pieces (natural size).

25° to 26° C. in an electric oven. The sand used in this work was washed successively with concentrated hydrochloric acid, tap water, and distilled water; it was then dried before use. About 23 ml. of dry sand were added to each Petri dish—an amount just sufficient to take up by capillarity 10 ml. of distilled water (or the solution to be tested). Under these conditions buds and roots first appear in about six days, and by nine days the maximum

number is formed. Observations were usually made after ten days with the aid of a dissecting microscope (fig. 3).

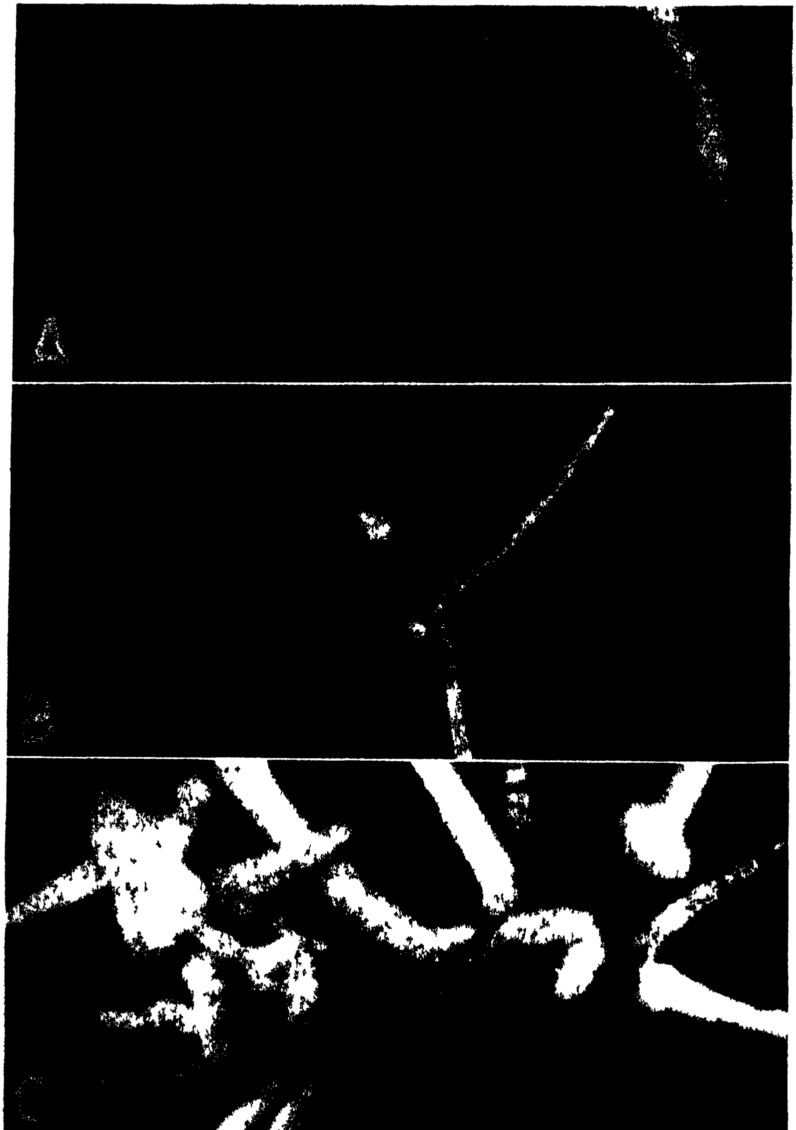


FIG. 3. Regeneration of test pieces. A: test piece at time of preparation. B: normal regeneration from test piece 3 mm. in length showing 2 roots and 3 buds. C: test piece treated with a 1 per cent. lanolin mixture of naphthyleneacetic acid showing formation of numerous roots (×15).

Since a bud or root is discernible at the surface of the test piece as soon as it becomes organized, the number of buds and roots formed may be used as a quantitative measure of regeneration. The number produced per ten test pieces was chosen as a standard measure, each Petri dish thus constituting a sample. Five replications or more were used, giving a population of at least fifty test pieces for any single determination. No attention was given to the relative size or vigor of an individual bud or root since only the factors involved in their initiation were being considered. The results reported in this paper were obtained from over 15,000 test pieces.

A major criticism of the method used was the lack of control of microorganisms. Most of the test pieces were able to resist invasion, however, if they were not damaged severely by the experimental treatment. Since the irregular surface of the piece made sterilization almost impossible, it was thought better to use a larger number of test pieces rather than introduce other variables in trying to obtain sterile cultures. Another problem was the introduction of chemical impurities in the sand which might affect the growth responses of the pieces. Such possible effects were minimized by thoroughly washing the sand with acid and water before use. No satisfactory substitute for sand was found that afforded adequate aeration and a good water supply. An additional problem was the past history of the material tested. If one were to grow the horseradishes under controlled conditions, take special precautions to see that they receive no injuries, and then rigorously select the roots, more uniform material would be obtained. Such a procedure is not feasible, and thus the best alternative appears to be the selection of roots of about the same size and the use of only those root traces that appear to be comparable. At any rate, it is not the actual number of buds and roots produced in any one test that is significant; it is the general trend of an experiment which results from a large total number of tests.

TABLE I

RELATION OF AREA OF TEST PIECE TO AMOUNT OF REGENERATION

DIAMETER	AVERAGE NUMBER OF ROOTS PER TEN PIECES	AVERAGE NUMBER OF BUDS PER TEN PIECES
mm.		
3	16 ± 2*	33 ± 2*
6	19 2	32 2
9	17 2	35 2
12	39 3	70 4
15	37 3	67 4

* Standard deviation expressed to nearest whole number.

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$$

Experimentation

As a preliminary experiment, it was essential to determine the effect of the diameter of the test piece on initiation of buds and roots. Pieces 3 mm. long, varying in diameter from 3 mm. to 16 mm., were tested in the dark at 25° C. Owing to the distribution of the root traces in the root, only one root trace was included per test piece in pieces up to 9 mm. in diameter; above 9 mm. two root traces had to be included. The results, shown in table I, tend to indicate that only the root traces are important in bud and root initiation. For convenience in handling, the 6-mm piece was chosen as the standard piece.

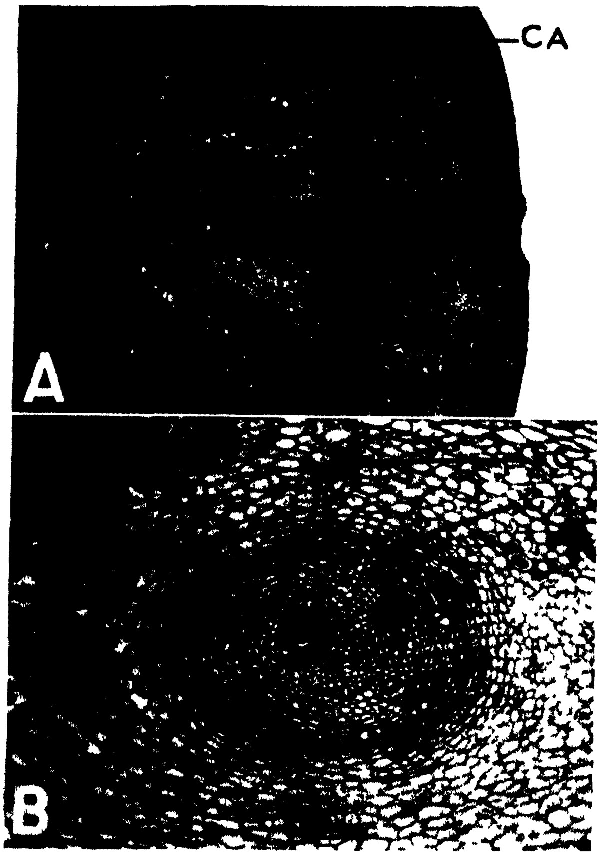


FIG. 4. Tissues associated with test piece. A: cross section of root 10 mm. in diameter showing longitudinal section of root trace. Diagram of test pieces imposed and drawn to scale as if the root were 24 mm. in diameter; ca, cambium (×15). B: tangential section through phloem portion of root showing cross section of root trace; ca, cambium; sc, stone cell (×150).

It was also necessary to know the relation of length of test piece to amount of regeneration. Pieces 6 mm. in diameter, but of lengths varying from 1 to 12 mm., were tested in the dark at 25° C. Figure 4 shows the tissues involved in these tests and the results are shown in figure 5. Al-

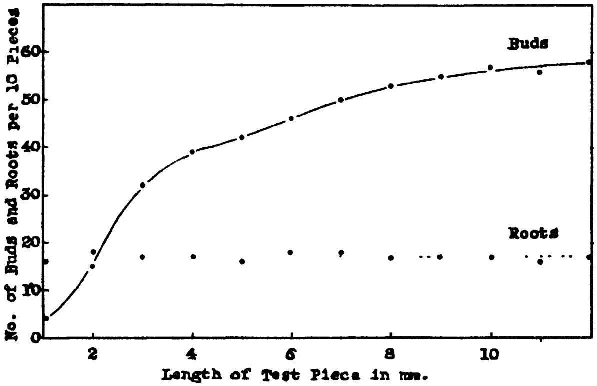


FIG. 5. Relation of length of test piece to amount of regeneration.

though the inner tissues apparently have no effect on the number of roots initiated, they do have a marked effect on the number of buds initiated.

Further evidence concerning the importance of the root trace for bud initiation was obtained by the following experiment. Pieces 6 mm. in diameter and 8 mm. long were used, and the root trace was severed by cutting a deep notch 1 mm. from the periderm. Others were ringed in the same place in order to sever nearly all tissue except the root trace. In all cases where the root trace was cut, the amount of regeneration corresponded to that of a piece 1 mm. long. On the other hand, when a root trace was left intact, the amount of regeneration corresponded to that of a piece 8 mm. long. In another experiment, pieces of various sizes were taken from parts of the root in which there were no root traces and tested in the dark at 25° C. In no case was there initiation of either roots or buds.

The question arose as to whether an inner portion of the test piece would initiate buds and roots if it were separated from the outer portion containing the periderm. Standard pieces 6 mm. in diameter and 8 mm. long were used; the outer 1 mm. consisting of periderm and outer phloem was discarded. In some cases the tissue produced a root or bud, more frequently a bud. If the outer 2-mm. piece was discarded, only buds were initiated, and these very rarely. These results seem to indicate that there is some factor, or factors, for both root and bud initiation in the outer 1 or 2 mm. of tissue.

It appears, therefore, that root and bud initiation are confined to the root trace. Only the outer tissues seem to be necessary for root initiation; for

maximum bud initiation, however, both inner and outer tissues seem to be necessary. Hereafter, for the sake of simplicity, the influence of the inner tissues in the stimulation of bud initiation will be referred to as the "bud factor." Furthermore, the term "factor" will be used in a broad sense to include any agent (physical, chemical, or otherwise) that produces an effect, and will not necessarily imply that a chemical entity is involved.

After the "bud factor" was shown to be confined to the root trace, it became desirable to know something about the rate of movement. Standard pieces 6 mm. long were divided into ten different lots. From one lot each day, the outer 1 mm. of tissue was removed from the pieces, and the inner 5 mm. were discarded. Observations were made on the fifteenth day. The results shown in figure 6 indicate that there is very little movement of the

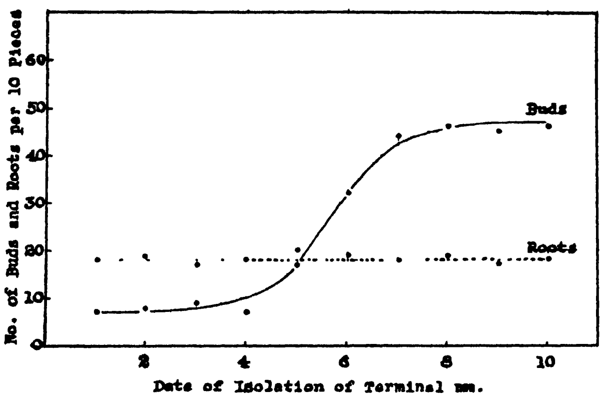


FIG. 6. Relation of time of isolation of 1 mm. test piece to amount of regeneration. The abscissa indicates time of isolation in days after beginning of experiment.

"bud factor" during the first four days of the experiment. Between the fifth and seventh days, however, there is a rather sudden movement. There are several ways of explaining these results. We may assume the "bud factor" to be present in the intact root, but we must then conclude that it does not move toward the periderm until at least four days after isolation of the test piece. On the other hand, the "bud factor" may not be present in the intact root and may be formed only about four days after isolation of the test piece. Another possibility is that the inner tissues merely act as a reservoir for the accumulation of bud inhibitors that are present in the outer tissues. These inhibitors may not move inward until after four days.

Since the "bud factor" has been shown to be transported along the root trace, some information regarding the diffusibility of this stimulus was sought. Standard test pieces 8 mm. long were used and the outer 1 mm. was removed with a clean, sharp razor. In some cases the two resulting pieces were immediately pressed tightly together, while in others a layer of

gelatin, agar, lanolin, or lens paper was placed between them. In every case the 1-mm. piece acted like an isolated 1-mm. piece. Apparently there was no diffusion of the "bud factor" across the break in continuity. These experiments were duplicated by attaching to the 1-mm. test piece a piece of inner tissue 7 mm. in length which had been killed with ether vapor (ether removed by aeration for six hours before pieces were used). In every case the 1-mm. test piece was killed, probably by the mustard oil or by other decomposition products of the dead tissue. There remained the possibility that if the "bud factor" were not present when the test piece was first isolated, it might not form unless it was under the influence of the developing primordia in the 1-mm. test piece. To test this possibility, the above experiments were duplicated, using test pieces that were severed 1, 2, 3, and 4 days after isolation. The same results were obtained, however, and in no case was there any evidence of bud stimulation. Thus one may conclude that the "bud factor" requires intact cells for its transfer.

Previous work has shown that when the horseradish root is cut into transverse segments about 4 cm. long, the development of buds and roots occurs in a polar manner; *i.e.*, buds develop at the morphological upper ends of the segments and roots at the morphological lower ends, regardless of the orientation of the segments during the period of regeneration (**13**). These observations were limited to gross development of both buds and roots and were not concerned with the actual initiation of growing points. Since there is this polarity in development, there might also be a polarity in the distribution of factors responsible for initiation. To test this possibility, transverse segments of the root, about 6 cm. long, were placed in a moist chamber on moist sand and maintained in the dark at 25° C. For nine successive days, standard test pieces, 6 mm. in diameter and 3 mm. in length, were removed from the tops and bottoms of these segments. Observations were made on the twelfth day. The results are shown in figure 7. The intact root appar-

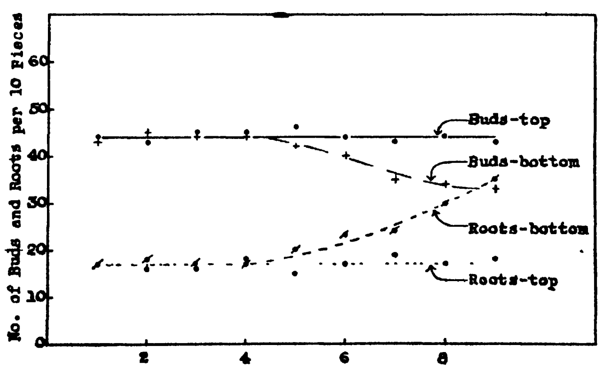


FIG. 7. Relation of polarity to initiation of buds and roots. The abscissa indicates time of isolation in days after beginning of experiment.

ently possesses no polarity in the distribution of the factors for bud and root initiation. The gradual increase in the number of roots and decrease in the number of buds, from the test pieces removed from the lower portions of the root segments, could be interpreted on the basis of the gradual accumulation in this region of some factor that stimulated root initiation and inhibited bud initiation. This factor might consist of the native auxins.

A partial analysis of the internal environment affecting bud and root initiation has shown that the inner tissues of the root trace stimulate bud initiation. This "bud factor" is transported along the root trace, is not diffusible, and is not polarly distributed. There are indications that it may be inactivated by one of the factors controlling root initiation.

Some of the relationships of the external environment to regeneration were next investigated. In order to determine the effects of variations in temperature, standard test pieces 6 mm. in diameter and 3 mm. in length were kept at various temperatures in the dark. Observations were made at the end of ten days. The results are shown in figure 8. The optimum

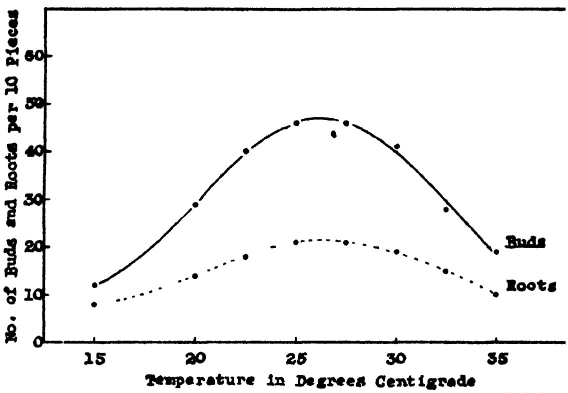


FIG. 8. Effect of temperature on bud and root initiation.

temperature for both root and bud initiation is about 26° C. Between 15° C. and 25° C. the Q_{10} for bud initiation is 2.8, while that for root initiation is 1.6. This may indicate that the limiting process in bud initiation is a chemical reaction, while that for root initiation is a physical one.

Additional information concerning temperature effects was obtained through the following experiment. Ten groups of test pieces were placed at 20° C. and 25° C. respectively. On each day, for a period of 10 days, a group was changed from 20° to 25° C. and another group from 25° to 20° C. Observations were made at the end of 12 days. The results are shown in figure 9. They indicate that temperature affects the initiation of buds and roots only at a particular stage in their development; *i.e.*, between the sixth and eighth days after isolation. Previous work (13) has shown

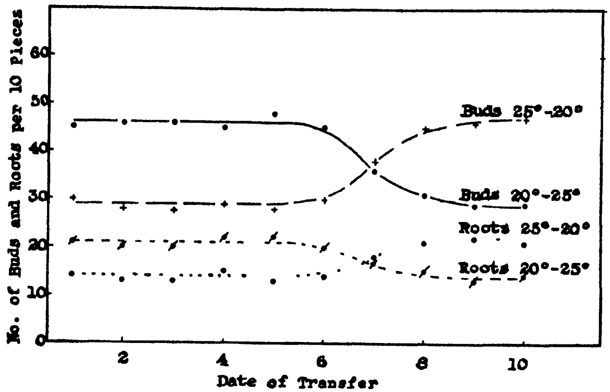


FIG. 9. Relation between time of temperature change and the initiation of buds and roots. The abscissa indicates time of transfer in days after beginning of experiment.

that this is the time when the meristematic regions are just beginning to organize into either buds or roots. It would appear, therefore, that temperature does not play an important rôle in the initial cell divisions, but is more effective in limiting the actual organizational processes.

A careful analysis of the effects of light on bud and root initiation was not attempted. A few experiments seemed to indicate that light stimulated bud production and inhibited root production. More exact experiments are necessary, however, to distinguish between the effects of light and the effects of temperature. To overcome any effects that light might have, all tests were made in the dark.

It was desirable to determine what effect the hydrogen-ion concentration of the medium around the test pieces would have on bud and root initiation. In these experiments, McIlvaine's standard buffer solutions (ranging from pH 2.2 to 8.0) were used instead of distilled water to moisten the sand in

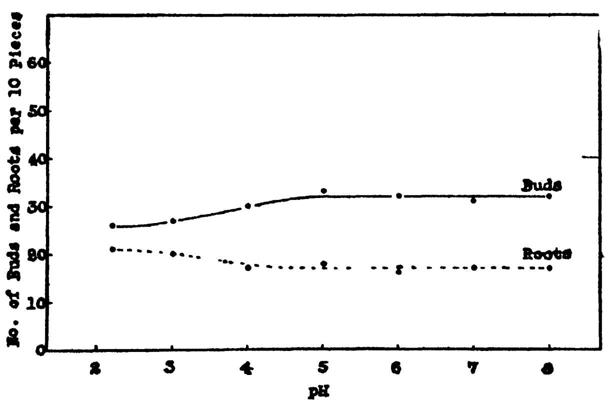


FIG. 10. Effect of hydrogen ion concentration on initiation of buds and roots.

the Petri dishes. These solutions (4), consisting of citric acid and potassium mono-hydrogen phosphate, are convenient to use because a wide range of pH may be obtained by varying the proportions of the two constituents. In every case the concentrations of the solutions were diluted one-tenth in order to prevent injury of the test pieces by high concentrations of salts. The hydrogen-ion concentration of the resulting solutions was checked with a quinhydrone electrode. No attempt was made to control the hydrogen-ion concentration of the tissue of the test pieces, and no determinations of tissue pH were made. The effects of these solutions on standard test pieces 3 mm. in length are shown in figure 10. Apparently the pH of the medium has little influence on the initiation of either buds or roots. This may be caused, in part, by a high buffer capacity of the tissue of the test piece. In the more acid solutions, there was a slight increase in number of roots and a slight decrease in number of buds. This could be accounted for most easily on the basis of an activation of native auxins by the acid (2).

After the method had been sufficiently standardized, it became of interest to determine the effect of specific chemical compounds on the initiation of buds and roots. In previous work, indoleacetic and naphthyleneacetic acids, in relatively high concentrations, were shown to be very effective in inhibiting the development of buds and in stimulating the development of roots (13). It was thus advisable to analyze further the effects of these compounds. Standard test pieces 3 mm. in length were treated with aqueous solutions of indoleacetic acid or naphthyleneacetic acid ranging in concentrations from 5.4×10^{-4} M to 5.4×10^{-14} M. Ten ml. of these solutions were added to the sand in Petri dishes. Since there were ten test pieces per dish, each piece had a maximum volume of 1 ml. of solution at its disposal, although only a small portion of this amount was probably absorbed. Lanolin mixtures of each of these substances were also applied to the cut surfaces of test pieces in concentrations ranging from 5.4×10^{-2} M to 5.4×10^{-7} M. These two different means of application gave essentially the same results. The effects of aqueous solutions of naphthyleneacetic acid are shown in figure 11. In the higher concentrations there is apparently a stimulation of root initiation and an inhibition of bud initiation (figure 3). No evidence was obtained for bud stimulation at any concentration over the wide range employed. The lower concentrations of naphthyleneacetic acid are more effective in bud inhibition than they are in root stimulation. Indoleacetic acid did not prove very effective, presumably because it was inactivated. The inactivation may have been caused by oxidizing enzymes from the test pieces, microörganisms, inorganic constituents of the sand, or a combination of these factors. At any rate, the "bud factor" appears to have no direct relation to the auxins; but it may be inactivated by them, or at least ineffective in their presence.

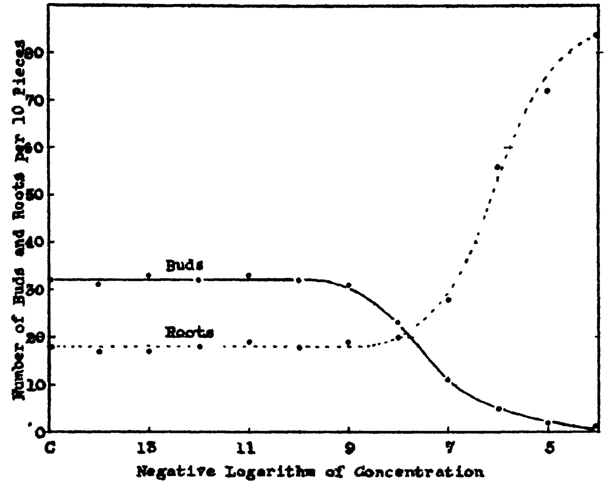


FIG. 11. The relationship of aqueous solutions of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates the concentration in grams per liter. C, control.

When small isolated pieces of various tissues of the root (xylem only, phloem only, periderm plus phloem, etc.) were treated with relatively high concentrations of naphthyleneacetic acid, roots were initiated from all of the tissues. The main cambial region and the region of the phellogen were the most active in this respect. Root traces were not included in any of these pieces.

A number of standard test pieces, 3 mm. in length, were divided into twelve lots. Each day, for 12 days, a different lot was treated with a 1 per cent. lanolin mixture of naphthyleneacetic acid. Each lot thus received but one application. Observations were made after 20 days. The results are shown in figure 12. Apparently the number of roots initiated is about the same regardless of the time of application of naphthyleneacetic acid. The number of buds, however, is most affected if the treatment is made during the first four days after isolation. The application of naphthyleneacetic acid after this time was apparently too late to inhibit the initiation. This period between 5 and 7 days is the time when meristematic areas are just beginning to organize into either a bud or a root. Thus after a bud is once organized, it will not change over into a root although root initials may develop at the base of the bud. Gross observations of buds from pieces treated 6 days and more after isolation substantiate this conclusion (compare the results of the experiment shown in figure 7 in this regard). Between the fifth and eighth days, in this experiment, there was a maximum decrease in the number of buds on the test pieces isolated from the lower portions of the segments. This might indicate that the native auxins (or other bud

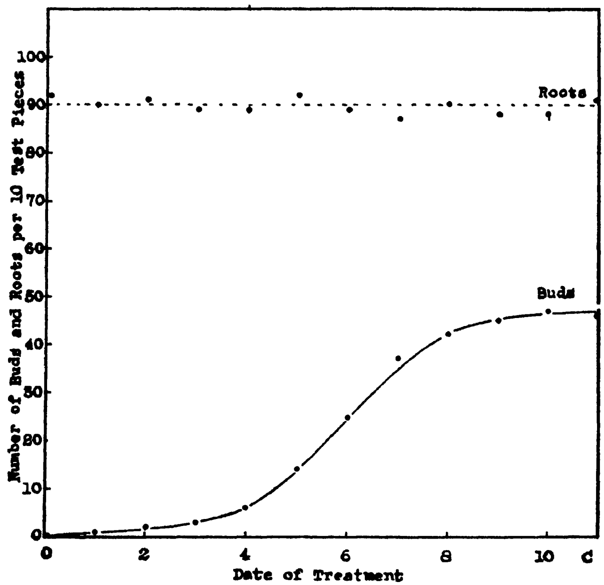


FIG. 12. The relation of time of application of a 1 per cent. lanolin mixture of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates time of treatment in days after start of experiment. C, control.

inhibitors) do not start to accumulate in the lower regions of transverse segments of the root until about 4 days after isolation.

In the experiments reported in table I and in figures 5, 6, 10, and 11, the maximum number of buds formed per ten test pieces, 6 mm. in diameter and 3 mm. in length, is near 32; while for the experiments reported in figures 7, 8, 9, and 12, the maximum number is near 46. This is attributable to the fact that horseradish roots from different sources were used for these two different groups of experiments. The higher maximum was obtained on test pieces from roots purchased in the fall; the lower maximum was obtained on test pieces from roots purchased in the winter. Thus it is shown, in an indirect fashion, that the past history of the root is an important factor in determining the amount of regeneration.

After some of the effects of auxin-like compounds had been ascertained, the effects of other pure substances were determined. A number of inorganic and organic compounds were applied over a wide range of concentrations as aqueous solutions to the sand in the Petri dishes. Standard 1-mm. or 3-mm. test pieces were used in testing these substances at 25° C. in the dark. The following is a list of the materials tried:

Ammonium sulphate	Calcium nitrate
Ascorbic acid	Chloral hydrate
Calcium chloride	Citric acid

Cupric sulphate
Dioxane
Eosin
Ethyl alcohol
Ethylene
Ethylene chlorohydrin
Ferric sulphate
Ferrous sulphate
Formaldehyde
Glycine
Hematoxylin
Hydrogen peroxide
Inositol
Magnesium sulphate
Maleic acid
Manganous sulphate
Methyl amine
Methylene blue
Nicotinic acid
Pangestin
Papain
Potassium chloride
Potassium cyanide
Potassium di-hydrogen phosphate
Potassium nitrate
Potassium permanganate
Saliva
Sodium taurocholate
Theelin
Theelol
Thiourea
Tyrosine
Urea
Urine
Vitamin B_1 (thiamin)
Vitamin B_2
Yeast extract (aqueous)
Zinc sulphate

In general, high concentrations were toxic, but other than this, no marked effects were produced. Thus it is probable that none of these substances alone limited the initiating processes.

Preliminary extracts from various tissues of the horseradish root have given some indication of being capable of stimulating bud initiation. These extracts were tested on standard 1-mm. pieces and at least four different concentrations of each extract were used; the highest concentration was usually toxic. The following is an outline of the extracts tried:

I. Inner tissues
  1. Ether extract
  2. Cell sap
II. Outer and inner tissues
  1. Water extract
  2. Fraction of water extract soluble in 70 per cent. alcohol
  3. Fraction of water extract insoluble in 70 per cent. alcohol
III. Inner tissues from roots allowed to regenerate 6 days
  1. Ether extract
  2. Water extract
  3. Cell sap
  4. Water diffusate
  5. Water extract[2] following previous ether and water extracts

[2] These extracts stimulated bud initiation at least 300 per cent.

6. Cell sap from frozen tissue
   a. Soluble fraction not passing through collodion membrane
   b. Insoluble fraction not passing through collodion membrane

IV. Outer tissues from roots allowed to regenerate 6 days
   1. Ether extract
   2. Water extract[2] following previous ether extract
   3. Cell sap from frozen tissue
      a. Soluble fraction not passing through collodion membrane
      b. Insoluble fraction not passing through collodion membrane

V. Outer and inner tissues from roots allowed to regenerate 6 days
   1. Water extract
   2. Fraction of water extract soluble in 70 per cent. alcohol
   3. Fraction of water extract insoluble in 70 per cent. alcohol

One encounters many difficulties in trying to obtain active extracts since there may be many different types of inhibitors present, and inactivation of an extract may take place for many reasons. The results, however, indicate that the "bud factor" is a chemical entity. This may be more apparent than real since the extracts may be effective by destroying bud inhibitors (such as the auxins) and may be entirely independent of the "bud factor." Further work of this type is now in progress.

Discussion

From the experiments reported here, it is apparent that the various tissues of the horseradish root possess different capacities for influencing the regeneration processes. Buds and roots are initiated only in association with a lateral root trace. The outer portions of the root trace appear to be responsible for root initiation; for maximum bud initiation, however, both the inner and outer portions are necessary. PRIESTLEY and SWINGLE (15) review some work on sea kale in this regard. The xylem tissues of the root of this plant, when isolated, produce buds from any of the cut surfaces, but do not form roots. The extra-cambial tissue, on the other hand, produces roots, but no buds. Thus these two species of plants are similar in that the inner tissues contain factors for bud initiation, and the outer tissues, factors for root initiation. They differ in that the horseradish regenerates only in association with a root trace, while any of the tissues of the sea kale root may regenerate. Furthermore, the horseradish requires factors that are present in the outer tissues of a root trace for maximum bud initiation.

The analysis of polarity phenomena in the horseradish indicates that in the dormant root there is no polar distribution of factors that cause either root or bud initiation. If the root is placed in an environment favorable for regeneration, however, the factors causing root initiation seem to become

polarly distributed. The factors associated with bud initiation also apparently become polarly distributed; this might be interpreted as caused by the inactivation of bud-initiating factors in the lower portions of the root or its isolated segments. SCHWANITZ (18) has done some work on this subject. He reports that intact rhizomes of *Lathyrus pratensis* and *Agropyrum repens* show a marked polar development of buds when allowed to regenerate. If the rhizomes are cut into transverse segments at the beginning of the experiment, each piece produces as many buds as any other piece. If severed on successive days, a gradual establishment of polarity is shown by the differential amount of bud development of the transverse segments. Further evidence has been accumulated by JONES (11), who reports that when segments of sea kale roots 5 to 10 cm. in length are centrifuged at least 3 days (1000 r.p.m.) with the apex of the segments away from the center, buds are usually produced from the base of the segments as well as at the apex. If segments are centrifuged with the base away from the center, however, they regenerate in a normal manner, *i.e.,* buds at apex and roots at base. This might mean that certain bud-inhibiting substances (such as auxins) are not subject to polar distribution when under the influence of an opposing centrifugal force, and thus their diffuse distribution allows buds to form from the basal ends of the segments. JONES also reports that segments of sea kale roots, 2 mm. or less in length, produced buds on both ends, while longer segments produced buds only on the upper end. This might indicate that the longer segments have more bud inhibitors than the shorter. STOUGHTON and PLANT (20), using segments of sea kale roots 7 cm. in length, cut off 1 mm. of tissue from the base and apex every 5 days for a period of 8 weeks. They obtained buds on both ends of 25 per cent. of the segments. This work would indicate that bud-inhibiting substances were removed when the end tissues were removed. FISCHNICH (6) was able to produce buds from callus at the basal end of poplar cuttings, that would ordinarily have produced roots, by ringing the cutting above the callus. The ringing supposedly prevented the accumulation of auxins in the basal callus.

In the horseradish, temperature has more effect on bud initiation than on root initiation. Other workers have reported similar results in regard to the relation of temperature to bud growth. CLARK (3) has shown that the decapitated hypocotyls of seedling tomato plants do not form buds from the cut surface unless the temperature is above 20° C. SIMON (19) reported that the development of buds from the callus of poplar cuttings was better at 32° C. than it was at 26° C. A temperature of 18° C. resulted in the formation of very little callus and inhibited the development of buds. JONES (11) found that a local increase of temperature as little as 2° C. induced the formation of buds on the lower ends of sea kale segments where roots ordi-

narily would have developed. Temperature is thus an important factor in bud initiation.

The hydrogen-ion concentration of the medium had little effect on bud and root initiation in the horseradish. JONES (11) reports similar results for sea kale. BONNER's analysis (2) of the effects of hydrogen ions on the Avena coleoptile shows that the effects of acids are produced through the activation of auxins. RIEHM's results (17) are a little difficult to understand in this regard. When he floated leaf fragments of *Cardamine pratensis* on a solution of KH_2PO_4, buds were produced in 3 days and roots in 5 days. When the leaf fragments were floated on K_2HPO_4, however, roots were formed in 3 days, but buds were not formed until after 14 days.

In the horseradish, the application of naphthyleneacetic acid over a wide range of concentrations inhibits the initiation of buds and stimulates the initiation of roots. STOUGHTON and PLANT (20) obtained similar results with sea kale roots by using relatively high concentrations of indoleacetic acid. WENT (24) also reports similar results with the roots of *Oenothera macrosiphon.* Thus it seems that, in certain plants at least, the initiation of buds from roots is inhibited by auxin-like compounds, while the initiation of roots is stimulated. The effects of these compounds on bud initiation from stems, however, are not so consistent. LINK and EGGERS (14) report inhibition of bud initiation from decapitated flax hypocotyls by application of relatively high concentrations of indoleacetic acid. On the other hand, GREENLEAF (8) reports a stimulation of bud initiation from the callus of decapitated tobacco plants through the use of a 1 per cent. indoleacetic acid mixture in lanolin. Likewise, GOLDBERG (7) reports similar results for cabbage seedlings. BEAL (1) obtained axillary buds by treating decapitated stems of *Lilium harrisii* with relatively high concentrations of indoleacetic acid. A closely related species, *L. longiflorum,* produced only roots in response to the treatment. HOWARD (10) reports that decapitated kale plants produce both buds and roots when treated with indoleacetic acid, but the buds are formed later than the roots—presumably after the supply of indoleacetic acid is exhausted. The work of COOPER (5), STUART (21), and WENT (23) on the mobilizing action of auxin-like compounds may lead the way to a solution of these problems in the future.

Ascorbic acid, thiamin, ethylene, and other "active" compounds are not effective in stimulating the initiation of either buds or roots in the horseradish. Extracts, however, indicate that the "bud factor" is a chemical entity. A factor affecting the development of buds has been reported by WENT (22). It is probably formed in the roots, requires intact cells for its transfer, and functions in bud elongation in collaboration with auxin. WENT has named this factor "caulocaline." Whether this factor is involved in bud initiation is not known.

Summary

1. A method of analyzing some of the factors associated with root and bud initiation, by the use of small isolated pieces of the horseradish root, is outlined. The results from more than 15,000 test pieces are reported.

2. The various tissues of the horseradish root apparently possess different capacities for influencing the regeneration processes. Root and bud initiation are confined to the lateral root traces. The outer tissues of the root trace are apparently responsible for root initiation; for maximum bud initiation, both outer and inner tissues are necessary.

3. The factor (or factors) in the inner tissues that stimulates bud initiation moves only along the root trace, and requires intact cells for its transfer.

4. The dormant root of the horseradish does not possess a polar distribution of either root or bud initiation factors. A polarity in root initiation is established only after the root is placed in a favorable environment for regeneration. An apparent polarity in bud initiation is established at the same time.

5. Variations in temperature have more effect on bud initiation than on root initiation. Between 15° C. and 25° C. the Q_{10} for bud initiation is about 2.8; that for root initiation is about 1.6. The optimum temperature for both bud and root initiation is about 26° C. Temperature produces its greatest effect just at the time the meristematic regions are organizing into buds or roots.

6. The hydrogen-ion concentration of the medium has little effect on either root or bud initiation.

7. Naphthyleneacetic acid, over a wide range of concentrations, inhibits bud initiation and stimulates root initiation. At no concentration is there any evidence of the stimulation of bud initiation. Indoleacetic acid was not very effective, presumably because it was inactivated. Relatively high concentrations of these substances are capable of inducing root initiation from isolated pieces of any living tissue of the root.

8. Numerous organic and inorganic compounds, including various vitamins and other "active" substances, had little effect on either root or bud initiation.

9. Tests with extracts of various parts of the horseradish root indicate that one of the factors present in the inner tissues that stimulates bud initiation is a chemical entity.

The writer is indebted to members of the Department of Botany of the University of Chicago for helpful suggestions made during the course of this study.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BEAL, J. M. Histological responses of three species of Lilium to indoleacetic acid. Bot. Gaz. **99**: 881–911. 1938.
2. BONNER, JAMES. The relation of hydrogen ions to the growth rate of the Avena coleoptile. Protoplasma **21**: 406–423. 1934.
3. CLARK, FRED R. Temperature as a possible factor in regeneration. Plant Physiol. **6**: 196. 1931.
4. CLARK, W. M. The determination of hydrogen ions. Williams and Wilkins, Baltimore. 1928.
5. COOPER, WILLIAM C. Transport of root-forming hormone in woody cuttings. Plant Physiol. **11**: 779–793. 1936.
6. FISCHNICH, O. Die Rolle des Wuchstoffes bei der Bildung von Adventivsprossen und -wurzeln. Ber. d. bot. Ges. **56**: 144–152. 1938.
7. GOLDBERG, ETHEL. Histological responses of cabbage plants grown at different levels of nitrogen nutrition to indole(3)acetic acid. Bot. Gaz. **100**: 347–369. 1938.
8. GREENLEAF, WALTER H. Induction of polyploidy in Nicotiana. Science n.s. **86**: 565–566. 1937.
9. HOLM, THEODORE. On the development of buds upon roots and leaves. Ann. Bot. **39**: 867–881. 1925.
10. HOWARD, H. W. Possible action of phytohormones as root-determiners. Ann. Bot. **2**: 933–942. 1938.
11. JONES, W. N. Polarity phenomena in sea kale roots. Ann. Bot. **39**: 359–372. 1925.
12. KRENKE, N. P. Wundkompensation, Transplantion und Chimären bei Pflanzen. Transl. by N. BUSCH and O. MORITZ. Julius Springer, Berlin. 1933.
13. LINDNER, ROBERT C. Effects of indoleacetic acid and naphthyleneacetic acids on development of buds and roots in the horseradish. Bot. Gaz. **100**: 500–527. 1939.
14. LINK, GEORGE K. K., and EGGERS, VIRGINIA. Inhibition of adventitious bud initiation in hypocotyl of flax by indole-3-acetic acid and flax extract. Nature **142**: 398–399. 1938.
15. PRIESTLEY, J. H., and SWINGLE, CHARLES F. Vegetative propagation from the standpoint of plant anatomy. U.S.D.A. Tech. Bull. 151. 1929.
16. RAUH, WERNER. Die Bildung von Hypocotyl und Wurzelsprossen und ihre Bedeutung für die Wuchsformen der Pflanzen. Nova Acta Leopoldina **4**: 396–457. 1937.
17. RIEHM, E. Beobachtungen an isolierten Blättern. Zeitschr. Naturw. **77**: 281–314. 1905.

18. SCHWANITZ, F. Beiträge zur Analyse der pflanzlichen Polarität. Beih. bot. Centralbl. **54A**: 520–530. 1936.
19. SIMON, S. Experimentelle Untersuchungen über die Differenzierungsvorgänge im Callusgewebe von Holzwächsen. Jahrb. wiss. Bot. **45**: 351–478. 1908.
20. STOUGHTON, R. H., and PLANT, W. Regeneration of root cuttings as influenced by plant hormones. Nature **142**: 293–294. 1938.
21. STUART, NEIL W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. Bot. Gaz. **100**: 298–311. 1938.
22. WENT, F. W. Specific factors other than auxin affecting growth and root formation. Plant Physiol. **13**: 55–80. 1938.
23. ————. The dual effect of auxin on root formation. Amer. Jour. Bot. **26**: 24–29. 1939.
24. ————. Some experiments on bud growth. Amer. Jour. Bot. **26**: 109–117. 1939.

THE ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

JAMES H. C. SMITH

(WITH ELEVEN FIGURES)

Introduction

In the process of photosynthesis as carried out by the higher plants, the leaf absorbs carbon dioxide from the air and transforms it into organic matter. This assimilation of carbon dioxide is accomplished through a number of intermediate steps and any complete analysis of the mechanism of this process requires that the carbon be traced through these consecutive steps. Obviously the first step is the absorption of carbon dioxide from the air.

The critical experiments of BLACKMAN (4) demonstrated that the carbon dioxide of the air enters the leaf through the stomata. At first this view was difficult to accept because it seemed impossible that the volume of carbon dioxide necessary to maintain active photosynthesis could diffuse through such a small area. The stomatal area in the sunflower leaf is only 3.75 per cent. of the total.

This difficulty was removed, however, when BROWN and ESCOMBE (5, 6) showed that the diffusion of a gas through a perforated septum is almost unobstructed if the apertures have the proper dimensions and distribution. Examination showed that the required conditions are fulfilled by the stomata of the sunflower leaf, and that the amount of carbon dioxide necessary to maintain the maximum rate of photosynthesis so far observed can easily diffuse into the leaf provided "the interior of the leaf were a perfect 'sink' of atmospheric carbon dioxide." These small openings have the power of "drinking in atmospheric carbon dioxide" about fifty times faster than apertures of like dimensions filled with "a constantly renewed solution of alkali hydroxide."

This exceptional ability to "drink in" carbon dioxide led WILLSTÄTTER and STOLL (33a) to search for the carbon dioxide absorptive agent in leaves. Their researches (33b) showed that some leaves, particularly sunflower and nettle leaves, even when unilluminated, absorb reversibly considerable quantities of carbon dioxide. This absorption is not a function of the life process of the leaves. Probably the green pigments of the leaf have nothing to do with this reaction because yellow varieties of certain species absorb carbon dioxide as well as the green varieties. WILLSTÄTTER and STOLL (33c) isolated no substances from leaves which could account for the carbon dioxide combining capacity. They suggested that alkali and alkaline earth bicarbonates, especially magnesium bicarbonate, might be the source of the

reversible absorption. They were more inclined to the view, however, that the chief absorptive agents are the amino compounds which react to form carbamino acids.

WILLSTÄTTER and STOLL (33d) also measured the absorption of carbon dioxide by chlorophyll both in true solution and in colloidal suspension. From these measurements they concluded that chlorophyll, molecularly dispersed in alcohol, absorbed no carbon dioxide but that when colloidally dispersed in water it absorbed reversibly a small but definite quantity of this gas in addition to that absorbed by the magnesium which was split from the pigment as magnesium bicarbonate. They performed experiments to determine whether the carbon dioxide absorbed by the chlorophyll could be reduced to formaldehyde with the simultaneous production of a peroxide (33e). In every case the results were negative and the conclusion was reached that the illumination of chlorophyll in an atmosphere of carbon dioxide was not sufficient of itself to produce photosynthesis (33f).

In view of the importance of carbon dioxide absorption to photosynthesis and the fact that the active agent for the absorption of carbon dioxide by leaves had not been identified, SPOEHR and MCGEE (24, 25, 26) undertook to establish the nature of the absorbing substance in leaves. Their experiments demonstrated that dried leaf material absorbed carbon dioxide; that the chlorophyll could be extracted with acetone without affecting the absorption; that an absorbing agent could be extracted from dried sunflower leaves by means of water saturated with ether; and, that the dissolved material maintained quantitatively the absorption capacity removed from the leaf material. Because of the removal of the absorptive material from leaves by the CHIBNALL-SCHRYVER method for protein extraction (water saturated with ether) SPOEHR and MCGEE were inclined to the view that a proteinaceous substance was responsible for this absorption through the carbamino reaction.

Further investigations by SPOEHR and NEWTON (27, 28) established that the absorptive material could be precipitated from the water extract of sunflower leaves by the addition of alcohol, and that this material was diffusible through an animal membrane. This material did not contain enough amino-nitrogen or total nitrogen to account for the absorption of carbon dioxide in equivalent molecular proportions. Consequently the hypothesis of the carbamino reaction as the source of the absorption was no longer tenable. These experiments also showed that "the larger part of the absorption of carbon dioxide by dried leaf material and the alcoholic precipitates obtained therefrom could be ascribed to bicarbonate formation."

Comparison showed that the leaves from sunflower and nettle possessed the highest carbon dioxide absorptive capacity of all the leaves studied. In fact dried leaf material from spinach, hydrangea, turnip, alfalfa, rhu-

barb, and grass absorbed very little more carbon dioxide than could be accounted for by the water added. The final conclusion of SPOEHR and NEWTON was that possibly all leaves possess an absorptive capacity for carbon dioxide but that certainly for many it is very small.

Later SPOEHR (**23**) pointed out that special experiments would be required to demonstrate whether or not this absorption had any connection with the photosynthetic process, but that it is suggestive that both sunflower and nettle leaves possess high absorptive capacity for carbon dioxide and high photosynthetic activity.

In spite of the attempts made to establish the chemical system whereby carbon dioxide is absorbed by certain leaves, no definite knowledge of the constituents responsible for this reaction had been gained; nor had it been determined whether this reaction was in any way related to the photosynthetic process. Because of the importance of these questions, both explicit and implied, renewed attempts have been made to analyze the process whereby carbon dioxide is absorbed by the unilluminated leaf (**29**). For this purpose the carbon dioxide absorptive capacity of living and killed leaves, of chlorophyllous and nonchlorophyllous leaves, and of petals, roots, and leaves have been compared. The absorption of carbon dioxide by fractions (differing in solubility) from the sunflower leaves and by the chemical substances obtained therefrom has also been measured.

From these measurements it has become evident that leaves possess a very complex system for the absorption of carbon dioxide. This system is composed of several interdependent chemical reactions which are in equilibrium with the carbon dioxide of the atmosphere. This system might provide a reservoir of carbon dioxide which would be available to the photosynthetic process.

Experimental procedure

APPARATUS FOR MEASURING THE AMOUNT OF CARBON DIOXIDE ABSORBED

The apparatus used for measuring the absorption of carbon dioxide (fig. 1) consisted of two parts: I, a manometric system for measuring the absorption of the gas; and II, a pumping system for removing, collecting, and analyzing the gas from the reaction vessel.

ABSORPTION APPARATUS (I).—A gas reservoir G could be filled with the gas whose absorption was to be measured. After evacuation of G by means of a Hyvac or a mercury diffusion pump through stopcocks B and H, it was filled from a gas tank attached through stopcock I. The pressure in the reservoir G was measured by manometer F. Manometer D was included so that the pressure in reservoir G could be measured without altering its volume. Manometer D indicated when the pressures in G and F were equal. Equality of pressures in G and F was obtained by evacuation

through H and adjustment of the height of the levelling-bulb attached to A. Capillary tubes C and E served as safety tubes for the evacuation of manometer D. Capillary tube J connecting reservoir G and reaction flask L was long enough to permit the shaking of L. Stopcocks K and M served to connect the reaction flask either to the reservoir G or to the pumping system

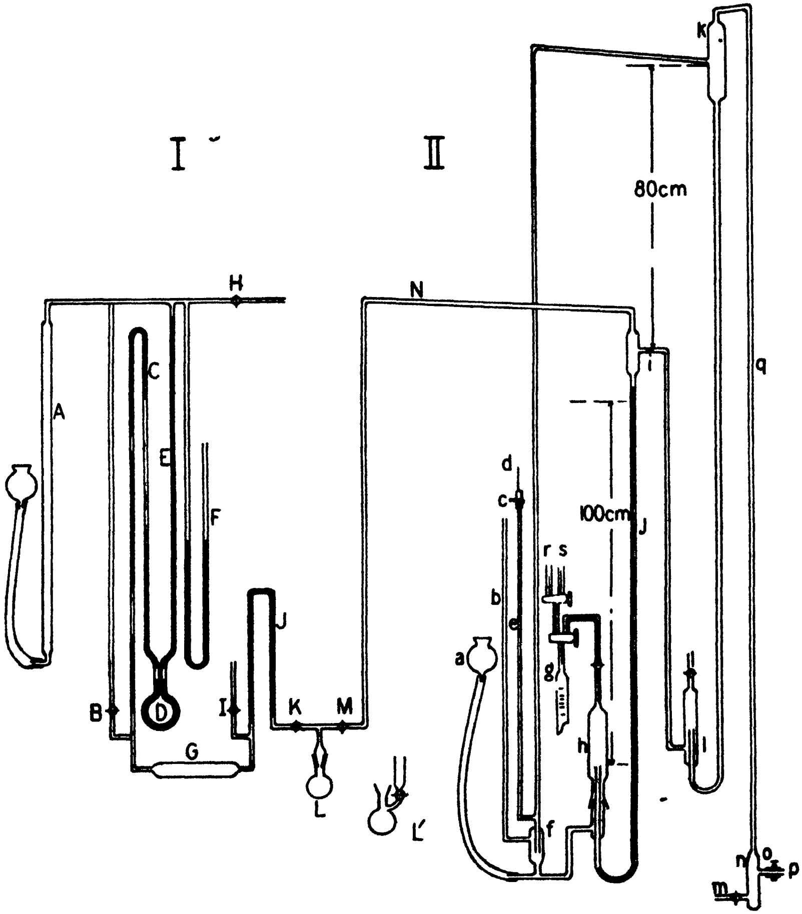


FIG. 1. Apparatus for measuring the absorption of carbon dioxide by leaf material. (I), absorption system. (II), pumping and gas-analysis apparatus.

(II). Reaction flask L′ equipped with an addition funnel could be substituted for reaction flask L when desired.

The gas reservoir G and the absorption flask L were submerged in a constant-temperature water bath. All tubing, C and J, outside of the water bath and included in the gas-measuring system was capillary tubing. This comprised about 3 per cent. of the total volume. Approximately a 0.1 per

cent. change in volume was caused by a change of 10° C. in room temperature. Under the experimental conditions used, error from this source was negligible.

APPARATUS FOR COLLECTING AND ANALYZING THE GAS (II).—The gas was pumped from the absorption flask L by means of a Sprengel pump, collected in a gas reservoir *h*, and transferred to a Hempel gas-analysis apparatus *g* for analysis. Descriptions of the Sprengel pump are given in manuals of laboratory practice (**7**, **19**). The design shown here was better suited to our purpose than the ones more commonly used.

An air lift carried mercury from reservoir *f* to chamber *k*. The air lift was activated by a water aspirator attached at *p*. Screw clamp *o* regulated the flow of air to the aspirator. The proper mercury level was maintained in *f* by the levelling-bulb *a*. Screw clamp *c* regulated the flow of air through capillary *d*. The mercury, separated from the air in chamber *k*, flowed in a fine stream through nozzle *i* trapping the gas in capillary *j* and removing it from the absorption apparatus.

The gas receiver *h* was connected through a ground-glass joint so that it could be removed easily for cleaning. The air inlets to *f* were furnished with long tubes *b* and *e* so as to prevent the spilling of mercury when levelling bulb *a* was raised. The gas trap *l* was inserted to eliminate the sweeping of small extraneous gas bubbles into receiver *h*. Mercury which spattered into tube *q* was collected in receptacle *n* and removed through stopcock *m*.

Only a few critical dimensions needed to be regarded for the proper operation of the pump. Two of these are marked on the drawing. The distance between the nozzle *i* and the inlet into *k* had to be greater than the height of the column of mercury supported by the pressure in the tube N, which for our purpose was greater than 760 mm. The height of the column of mercury in the capillary tube *j* was also great enough to insure a sufficient rate of flow of mercury, in this instance a height of about 100 to 110 cm. The diameter of the capillary *j* (approximately 1 mm.) was sufficiently large to permit a rapid flow of mercury but small enough to prevent the trapped air from eddying past the flowing mercury. The opening of nozzle *i* had to be adjusted empirically to meet the other demands of the apparatus. This was done by constricting the tube to the desired diameter (approximately 1.5 mm.) by means of a hand torch.

The conversion factor for transforming pressure change in reservoir G to volume of gas absorbed in vessel L was obtained by measuring the change of pressure in reservoir G caused by removal of known volumes of gas. These volumes were measured in buret *g*. The reproducibility of these measurements was found to be about ± 0.1 ml.

Any change in the volume of the system KJGC required a redetermina-

tion of the value for the conversion factor. The value of this factor was not dependent on the volume of the reaction flask L. The reaction vessels were approximately 50-ml. capacity and the volume of KJGC was 122.7 ml. in many of the experiments reported.

PROCEDURE.—A standard procedure was adopted for measuring the absorption of carbon dioxide by the various materials which were placed in the reaction flask L, or L′. The flask was attached to the apparatus through the ground-glass joint and the material allowed to come to temperature equilibrium with the bath. Living leaves were stored an additional length of time, one or two hours, before measurements were begun so as to lessen their respiration.

Next, the flask was evacuated by means of the Sprengel pump. For nonliving material the pumping was discontinued when gas bubbles ceased to appear in the capillary tube *j*. This point was easily determined. For living material, however, gas continued to be removed even after long periods of pumping. For such material an arbitrary period of twenty minutes was set, since this had been shown to be ample time to remove all gas from the empty reaction vessel.

The space in the reaction vessel, not occupied by the plant material, was estimated by admitting nitrogen gas from the reservoir and noting the change of pressure in the reservoir. The volume of nitrogen taken into the reaction vessel was calculated from the change in pressure. Subtraction of the amount of nitrogen aborbed by the water of the leaves (assuming the absorption to be the same as for pure water) gave the free space in the reaction vessel. The nitrogen was then pumped from the reaction vessel into receiver *h*, transferred to buret *g*, measured, and analyzed for carbon dioxide. The volume of nitrogen obtained in this way usually agreed with the volume absorbed (estimated manometrically) within $\pm$ 0.2 ml.

The absorption of carbon dioxide was then measured in exactly the same manner. The absorption by living leaves was very rapid, ten minutes being sufficient for saturation. In contrast to this, complete absorption by killed leaves required several hours.

By making a series of measurements at increasing pressures of carbon dioxide, the relation between the absorption of carbon dioxide and its partial pressure was obtained.

When the absorptions were completed, the gas was collected in receptacle *h* and the carbon dioxide determined. The agreement between the volumes of gas measured manometrically and volumetrically demonstrated the reversibility of the absorption process.

Diffusion of the water vapor from the reaction vessel through the tube J into the reservoir G was prevented by keeping stopcock K closed most of

the time. It was opened frequently for very short intervals to establish equilibrium between the two vessels.

At equilibrium the partial pressure of the gas being absorbed was equal to the gas pressure in reservoir G less the vapor pressure of the material in the reaction flask. With leaves in the reaction vessel, this vapor pressure was taken as the vapor pressure of pure water (12.8 mm. at 15.0° C.).

When the carbon dioxide was to be liberated from leaves by the addition of acid the reaction vessel L′ was always used. In these instances the absorption measurements were carried out as has been described, the carbon dioxide was pumped off as completely as possible, and 10 ml. of 6 N hydrochloric acid containing one drop of heptyl alcohol was added from the addition funnel. The gas which was liberated was collected in the receiver *h* and the total volume of carbon dioxide determined. During the evolution of the gas the reaction vessel was shaken continuously.

After an experiment had been completed the material was removed from the reaction flask and dried in an oven at 110° C. The water content thus obtained was used to estimate the amount of carbon dioxide dissolved by the water in the absorbing system, on the assumption that the water of the leaf absorbed the same quantity of carbon dioxide as pure water.

GAS-FLOW METHOD.—In a number of experiments (tables I and II) another type of absorption method was used. Instead of using pure carbon dioxide gas at various pressures, the absorptions were measured at various

TABLE I

ABSORPTION OF CARBON DIOXIDE BY LIVING LEAVES FROM DIFFERENT SPECIES OF PLANTS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

SAMPLE NUMBER	LEAF MATERIAL	CO_2 IN GAS	CO_2 ABSORBED	CO_2 CALC. FOR WATER	CO_2 EXCESS
		%	*ml.*	*ml.*	*ml.*
1	*Helianthus annuus*	98.5	10.20	8.01	2.19
2	*Malva parviflora*	100.0	11.93	7.77	4.16
3	*Libo cedrus*	99.7	7.66	5.01	2.65
4	*Eschscholtzia californica*	98.9	9.03	7.61	1.42
5	*Vicia sativa*	99.7	10.01	7.38	2.63
6	*Trifolium repens*	98.5	10.17	7.64	2.53
7	*Quercus douglasii*	99.7	6.38	5.56	0.82
8	*Nicotiana tabacum*	98.8	9.14	8.03	1.11
9	*Berberis aquifolium* (young leaves)	100.0	8.39	7.62	0.77
10	*Berberis aquifolium* (old leaves)	99.7	6.68	4.86	1.82
11	*Polypodium vulgare*	98.8	8.53	7.96	0.57
12	*Prunus cerasifera* var. *pissardii*	99.2	7.86	6.46	1.40
13	*Hordeum vulgare*	98.5	9.61	7.75	1.86
14	*Rosa* sp.	98.5	8.03	5.92	2.11
15	*Helianthus annuus* (normal)	98.2	10.40	7.46	2.94
16	*Helianthus annuus* (starved)	98.2	11.55	7.89	3.66

TABLE II

ABSORPTION OF CARBON DIOXIDE BY VARIOUS PLANT ORGANS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

SAMPLE NUMBER	PLANT ORGAN	PLANT SPECIES	CO_2 IN GAS	CO_2 ABS.	CO_2 CALC. FOR WATER	CO_2 EXCESS
			%	*ml.*	*ml.*	*ml.*
1	Petals	*Eschscholtzia californica*	99.6	8.23	8.24	–0.01
2	"	*Rosa* sp.	99.2	9.06	8.22	0.84
3	Coleoptile	*Hordeum vulgare*	99.7	9.72	9.00	0.72
4	Roots	" "	98.2	9.63	8.58	1.05
5	"	" "	98.8	9.30	8.30	1.00
6	Leaves (etiolated)	" "	98.8	9.90	8.60	1.29

partial pressures of carbon dioxide in nitrogen. The saturation was carried out by placing the leaves in reaction chamber G (fig. 2), and passing the

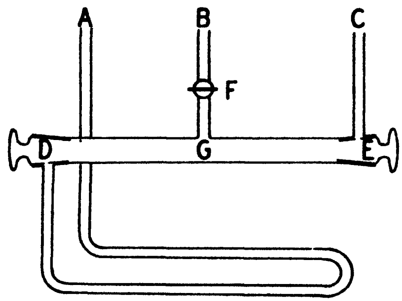


FIG. 2. Vessel for saturating leaf material with carbon dioxide at different partial pressures in a gas stream.

desired gas mixture through the chamber. The chamber G was connected through ground joints to the Sprengel pump at B and the gas supply at A.

Before saturation was begun, the chamber was closed off from tubes A and C by means of the perforated stoppers D and E, then evacuated by the Sprengel pump through the stopcock F. The reaction vessel was shut off from the Sprengel pump and gas was admitted to the chamber through the stopper D and allowed to flow through the chamber by opening stopper E. The end stoppers were then closed and the gas from the reaction vessel pumped into receiver *h*. The volume of the gas was measured in buret *g*. This volume minus the free space (obtained by a similar manipulation with nitrogen) gave the volume of carbon dioxide absorbed. The percentage of carbon dioxide in the gas phase at equilibrium was calculated from the expression

$$\frac{V_r - .V_a}{V_f} \times 100$$

where V_r is the volume of carbon dioxide in the gas pumped from the reaction vessel; V_a is the volume of gas absorbed; and V_f is the volume of the free space in the reaction vessel. $V_a = V_t - V_f$, where V_t is the total volume pumped out.

The carbon dioxide absorbed in excess of that attributable to the water was calculated from the equation, $V_e = V_a - V_w$. V_w is the product of the weight of water present in the leaves (grams) times the absorption coefficient of carbon dioxide in pure water. The values of V_e are shown in the last columns of tables I and II.

PREPARATION OF LEAF MATERIALS FOR ABSORPTION.—The fresh leaves after being harvested were washed with distilled water to clean off the surface dirt. The water remaining on the surfaces of the smaller leaves was removed by absorption in filter paper. Water was removed from the surfaces of the larger leaves by evaporation; the petioles were immersed in distilled water during this process.

The parenchymatous tissue of the larger leaves was removed from the main veins and cut into pieces approximately one square inch in area. Smaller leaves were used whole.

This material was weighed into the reaction flask and the carbon dioxide absorption measured. Ten grams (± 0.02 gm.) of material was used in all but a few cases where this amount of material was not available.

METHODS USED FOR KILLING LEAVES.—Leaves to be killed by freezing were harvested and washed in the manner already described. A 10-gm. sample was weighed into a small capped vial and immersed to the cap in a freezing bath of ethyl acetate and solid carbon dioxide. When the leaves had become frozen the tubes were removed and the leaf material allowed to thaw. This material was weighed into the reaction flask and used for absorption experiments.

The amount of water lost from the leaves during these manipulations was only about 1 per cent. Duplicate samples of leaves, one fresh, the other frozen, gave 14.9 and 15.5 per cent. dry weight, respectively.

Leaves to be killed by heating were placed into small capped vials and immersed in boiling water for from twenty to thirty minutes. The vials were allowed to cool before the contents were removed.

ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

Absorption of carbon dioxide by unilluminated leaves in excess of that ascribable to the water present is a property that is widespread among a number of species of plants (tables I and II). Only in one species so far examined was the amount of carbon dioxide *absorbed* less than that calcu-

lated for the water present and even in this case the volume of carbon dioxide *removed* by pumping was greater than that reckoned for the water. This plant, *Sedum praealtum,* has an acid sap, pH 4.08 (table III, no. 26).

Roots and coleoptiles of barley plants and the petals of roses also absorbed carbon dioxide in excess of the amount attributable to water. Petals from the California poppy (*Eschscholtzia californica*) were an exception (table II). At low pressures of carbon dioxide the absorption by these organs was more nearly proportional to the pressure than was the absorption of carbon dioxide by leaves.[1]

Starvation of leaves affected their ability to absorb carbon dioxide. Sunflower leaves which had been in sunlight all day and contained an abundant supply of starch (table I, no. 15) absorbed less carbon dioxide than similar leaves, cut from the same plants, which had been stored in the dark over night and had lost their starch (table I, no. 16). Leaves which lacked green pigments, however, absorbed carbon dioxide as shown in table III.

THE REVERSIBILITY OF THE CARBON DIOXIDE ABSORPTION

Carbon dioxide, to become available for photosynthesis, may have to dissociate from the substances by which it is first absorbed. This reversibility would then be an important property of the absorption system. For this reason the reversibility of the absorption process was measured for leaves of different species of plants and for different kinds of leaves from the same species.

The experiments were carried out in the apparatus shown in figure 1. The amount of gas absorbed, measured manometrically, was compared with the amount of gas that could be removed by pumping (table III, columns 6 to 8). Once the leaves had been treated with carbon dioxide the amounts of gas absorbed and removed were more nearly equal in subsequent treatments. This is shown by comparing the experiments lettered (a) with those lettered (b) and (c) in table III.

Complete reversibility was demonstrated for all the leaves examined, whether they were green, variegated yellow (no. 25), albino (no. 4), or etiolated (nos. 2, 9, 11, 16, 23). It is apparent from these results that the green pigment is not the principal factor involved in the reversible absorption of carbon dioxide by leaves. This reversibility is characteristic of killed leaves (table III, nos. 27–30) as well as of living leaves, consequently it is not dependent on some life process in the leaves.

In most instances the amount of carbon dioxide removed from living leaves was greater than that absorbed. This additional carbon dioxide undoubtedly came from the respiration of the leaves. Its production not only [illegible]ased the amount of carbon dioxide removable from the leaves but de-

[1] The results recorded in tables I and II were obtained by means of the gas-flow [illegible]od, (*cf.* fig. 2); those in table III, by apparatus, figure 1.

TABLE III

REVERSIBILITY OF CARBON DIOXIDE ABSORPTION BY LEAVES

Sample number	Leaf material	Fresh wt.	Dry wt.	Press. CO_2 at equil.	Vol. CO_2 absorb.	Vol. CO_2 removed	$\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$	Excess CO_2 abs.	Excess CO_2 rem.	Excess CO_2 abs. at 1 atmos.
	*Zea mays**	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
1	a Green	10.00	1.18	645.8	8.73	9.09	1.041	1.08	1.44	1.19
	b "	10.00	1.18	515.5	7.22	7.48	1.036	1.11	1.37	
2	a Etiolated	10.00	0.75	645.4	8.78	9.07	1.033	0.76	1.05	0.79
	b "	10.00	0.75	508.2	7.00	7.17	1.024	0.68	0.85	
3	a Green	10.00	1.18	632.1	8.39	8.75	1.043	0.90	1.26	1.05
	b "	10.00	1.18	517.4	7.10	7.26	1.023	0.97	1.13	
4	a Albino	10.00	0.86	681.5	9.68	9.94	1.027	1.32	1.58	1.38
	b "	10.00	0.86	517.9	7.80	7.72	0.990	1.45	1.37	
	c "	10.00	0.86	517.3	7.88	7.97	1.011	1.54	1.63	
5	a Green (water-rich)	10.00	1.16	653.8	9.03	9.18	1.017	1.27	1.42	1.38
	b " "	10.00	1.16	512.3	7.31	7.26	0.993	1.23	1.18	
6	a Green (water-poor)	4.96	1.15	627.1	4.33	4.33	1.000	1.12	1.12	1.32
	b " "	4.96	1.15	515.0	3.80	3.64	0.958	1.17	1.01	
	Phaseolus multiflorus									
7	a Green (water-rich)	10.00	1.40	708.4	9.42	9.80	1.040	1.25	1.63	1.29
	b " "	10.00	1.40	517.0	7.31	7.60	1.040	1.34	1.63	
8	a Green	4.49	1.32	678.3	3.61	3.82	1.058	0.73	0.94	0.77
9	a Etiolated	8.37	1.01	650.5	8.69	9.08	1.046	2.55	2.92	2.68
	b "	8.37	1.01	509.4	7.59	7.50	0.988	1.99	1.90	
	Pisum sativum									
10	a Green	10.00	1.17	650.7	9.01	9.99	1.111	1.30	2.28	1.43
	b "	10.00	1.17	510.2	8.27	8.37	1.012	2.19	2.29	
11	a Etiolated	10.00	1.13	658.8	9.50	10.29	1.083	3.49	4.28	3.66
	b "	10.00	1.13	485.9	8.39	8.52	1.015	4.20	4.33	

TABLE III—(*Continued*)

Sample number	Leaf material	Fresh wt.	Dry wt.	Press. CO_2 at equil.	Vol. CO_2 absorb.	Vol. CO_2 removed	$\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$	Excess CO_2 abs.	Excess CO_2 rem.	Excess CO_2 abs. at 1 atmos.
	Helianthus annuus	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
12	a Green (water-rich)	10.00	1.89	656.4	9.51	9.38	0.986	2.37	2.24	2.68
	b " "	10.00	1.89	512.1	7.79	8.10	1.040	2.22	2.53	
13	a Green (water-poor)	5.88	1.94	652.6	4.94	4.62	0.935	1.49	1.17	1.73
	b " "	5.88	1.94	453.3	3.65	3.59	0.984	1.25	1.19	
	Nicotiana tabacum									
14	a Green	10.00	3.26	650.1	6.85	6.75	0.985	0.97	0.87	1.03
15	a Yellow with age	10.00	0.81	705.9	8.73	8.71	0.998	0.03	0.01	0.08
16	a Etiolated	4.70	0.40	644.1	3.88	4.14	1.067	0.34	0.60	0.34
	Malva parviflora									
17	a Green (water-rich)	10.00	1.93	661.7	10.18	11.18	1.098	3.01	4.01	3.17
	b " "	10.00	1.93	314.1	6.20	6.58	1.061	2.80	3.18	
	c " "	10.00	1.93	503.5	8.77	9.20	1.049	3.32	3.75	
18	a Green (water-poor)	5.04	1.92	648.0	4.53	5.25	1.159	1.82	2.54	2.04
	b " "	5.04	1.92	277.7	2.79	2.91	1.043	1.63	1.75	
	c " "	5.04	1.92	507.8	4.26	4.43	1.040	2.14	2.31	
	Beta vulgaris									
19	a Green (water-rich)	10.00	1.62	642.0	7.59	7.84	1.033	0.37	0.62	0.43
	b " "	10.00	1.62	231.3	2.96	3.09	1.044	0.36	0.49	
	c " "	10.00	1.62	513.5	6.28	6.27	0.998	0.50	0.49	
20	a Green (water-poor)	6.29	1.59	651.6	4.43	4.29	0.968	0.32	0.18	0.36
	b " "	6.29	1.59	299.3	2.24	2.16	0.964	0.35	0.27	
	c " "	6.29	1.59	519.3	3.52	3.50	0.994	0.25	0.23	

TABLE III—(*Concluded*)

Sample number	Leaf material	Fresh wt.	Dry wt.	Press. CO_2 at equil.	Vol. CO_2 absorb.	Vol. CO_2 removed	$\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$	Excess CO_2 abs.	Excess CO_2 rem.	Excess CO_2 abs. at 1 atmos.
	Hordeum vulgare	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
21	a Green (water-rich)	10.00	0.94	647.1	8.57	9.64	1.125	0.70	1.77	0.75
	b " "	10.00	0.94	248.9	3.89	4.45	1.144	0.86	1.42	
	c " "	10.00	0.94	516.1	7.73	8.04	1.040	1.46	1.77	
22	a Green (water-poor)	6.74	0.91	662.1	6.10	6.63	1.087	0.92	1.45	0.93
	b " "	6.74	0.91	230.1	2.74	2.81	1.026	0.94	1.01	
	c " "	6.74	0.91	504.8	5.31	5.65	1.064	1.36	1.70	
23	a Etiolated	10.00	0.79	619.2	8.26	9.38	1.136	0.61	1.73	0.62
	b "	10.00	0.79	157.7	2.86	3.07	1.074	0.91	1.12	
	c "	10.00	0.79	490.3	7.46	7.46	1.000	1.40	1.40	
	Evonymus japonica									
24	a Green	10.00	4.50	664.1	5.73	7.58	1.323	0.83	2.68	0.86
25	a Yellow	10.00	2.54	668.6	8.11	9.30	1.147	1.42	2.61	1.54
	Sedum praealtum									
26	a	10.00	0.74	645.2	7.31	8.19	1.120	–0.72	0.16	–0.79†
	b " "	10.00	0.74	235.6	2.69	3.36	1.249	–0.24	0.43	
	c " "	10.00	0.74	505.9	6.05	6.58	1.088	–0.24	0.29	
	Helianthus annuus									
27	a Green (heated)	9.41	1.58	574.3	16.36	16.05	0.981	10.32	10.11	12.20
28	a Green (frozen)	9.84	1.74	663.6	21.20	20.54	0.970	13.99	13.33	14.43
29	a Green (ext. with H_2O)		1.33	670.1	16.48	16.47	0.999	4.15	4.14	4.20
30	a Green (ext. CO_2–H_2O)		1.24	679.5	11.59	11.42	0.985	0.11	–0.06	0.15

* Corn seedlings nos. 1 and 2 (variety Golden Bantam) were grown from the same lot of seed.

The corn seedlings (nos. 3, 4, 5 and 6) were grown from seed furnished by PROFESSOR A. C. FRAZER, Cornell University. Samples 5 and 6 were from the same lot of seed and grown under comparable conditions. The tobacco leaves (no. 16) were grown on fully developed tobacco plants which had been kept in the dark for some weeks. † An excess of 0.13 instead of a deficit of – 0.79 is obtained when the solubility coefficient for the water of the sap is substituted for that of pure water (*cf.* p. 200).

creased the absorption of an equivalent amount of carbon dioxide from the gas reservoir. This made the amount of gas absorbed too small (table III, column 9). For this reason it may be that the amount of gas removed by pumping (table III, column 10) is a better measure of the absorption than the gas uptake, measured manometrically. The amount of carbon dioxide removed from killed leaves was usually slightly less than the amount taken up. This deficit was reduced to an insignificant amount if the period of pumping was greatly prolonged; hours of pumping were often required to remove the last noticeable quantity of gas. Perhaps the cause of this was the slow diffusion of gas in the killed leaf.

One conspicuous difference observed between living and killed leaves (killed by either heating or freezing) was the rate at which each attained equilibrium with its surrounding atmosphere. Living leaves absorbed or evolved carbon dioxide very quickly and reached equilibrium within a very few minutes. Killed leaves, however, required hours to complete the reaction. This retardation in reactivity is probably caused by the breaking down of the structure of the leaf, which would make diffusion much slower. The retardation also might be caused by the inhibition of the chemical reactions with carbon dioxide.

Summarizing, it may be said that the uptake of carbon dioxide by the unilluminated leaf is strictly a reversible reaction which is independent of the presence of chlorophyll and of the life processes of the leaf.

ABSORPTION OF CARBON DIOXIDE BY DIFFERENT EXTRACTS FROM SUNFLOWER LEAVES

Preliminary to an investigation of what constituents in the leaf combine with carbon dioxide, the absorption and evolution (by acidification) of carbon dioxide by different extracts from sunflower leaves were determined. Frozen sunflower leaves were extracted with water and with water saturated with carbon dioxide. Sunflower leaves were chosen because of their large carbon dioxide absorption capacity, which has been remarked by others (**33b**, **28**). In order to obtain the extracts the leaves had to be killed. For this reason it was desirable to determine the effect of killing.

Examination showed that the leaves killed by freezing (– 70°) absorbed reversibly considerably more carbon dioxide (exclusive of that dissolved by the water of the leaf) than did living leaves (reversible CO_2, table IV, nos. 1 and 2). On the other hand, killed leaves evolved less carbon dioxide when treated with cold dilute hydrochloric acid (irreversible CO_2). When saturated with carbon dioxide at one atmosphere of pressure, the living and killed leaves contained approximately the same quantity of carbon dioxide (total combined CO_2).

The frozen sunflower leaves were extracted with water. Both the soluble constituents (no. 3) and the insoluble leaf residue (no. 4) absorbed

carbon dioxide (table IV, reversible CO_2), but only the insoluble residue liberated carbon dioxide on treatment with acid (nos. 3 and 4, irreversible CO_2). The total combined CO_2 in the residue was nearly double that in the dissolved material.

TABLE IV

THE DISTRIBUTION OF THE CARBON DIOXIDE COMBINED BY FRACTIONS OF SUNFLOWER LEAVES, SEPARATED BY DIFFERENCE IN SOLUBILITY. ALL VALUES RECORDED ARE BASED ON 10.00 GRAMS FRESH WEIGHT OF LEAVES. THE LEAVES USED IN NOS. 1 TO 6 WERE ALIQUOT PORTIONS TAKEN FROM THE SAME LOT OF LEAVES

SAMPLE NUMBER	LEAF MATERIAL	CARBON DIOXIDE COMBINED*		
		REVERSIBLE CO_2	IRREVERSIBLE CO_2	TOTAL COMBINED CO_2
		ml.	*ml.*	*ml.*
1	Living leaf	2.98	15.78	18.76
2	Frozen leaf	8.89	7.83	16.72
3	Water-soluble	4.82	− 0.31	4.51
4	Water-insoluble	4.88	4.28	9.16
5	CO_2-water-soluble	5.94	5.30	11.24
6	CO_2-water-insoluble	0.00	0.46	0.46
7	Leaf sap	2.42	(8.84) †	

* Reversibly combined CO_2. The amount of carbon dioxide absorbed by the leaf material, at a partial pressure of carbon dioxide equal to 1 atmosphere, in excess of the amount calculated to saturate the water present.

Irreversibly-combined CO_2. The amount of carbon dioxide that is liberated from the evacuated leaf material on treatment with cold dilute acid. In practice it is the amount of carbon dioxide obtained from the material (in equilibrium with a given partial pressure of carbon dioxide) when acidified, minus the amount of carbon dioxide absorbed by the leaf when the pressure of carbon dioxide is changed from zero to the equilibrium pressure.

Total combined CO_2. The sum of the reversible and irreversible carbon dioxide.

† This result was determined with the Van Slyke blood-gas analysis apparatus instead of the apparatus shown in figure 1 which was used for the other measurements.

The water-soluble substances were removed from the leaves (10.00 gm.) killed by freezing in the following manner. The sample of killed leaves was extracted thoroughly with three 100-ml. portions of distilled water. The solid residue was collected, rinsed with distilled water, transferred to the reaction flask, and its absorption measured (table IV, no. 4).

The extract was evaporated to dryness in a platinum dish on the water bath. The solid remaining was transferred quantitatively to the reaction flask by the use of distilled water and the absorption and evolution of carbon dioxide by this material measured (table IV, no. 3).

Although the water-soluble material failed to evolve carbon dioxide when treated with dilute hydrochloric acid, other experiments demonstrated that the leaf contains water-soluble substances which liberate carbon dioxide on acidification. It is probable, therefore, that these substances were decomposed with loss of carbon dioxide during the evaporation process.

The leaf residue, which was insoluble in water, was further extracted

with water saturated with carbon dioxide. The residue insoluble in this reagent (no. 6) did not absorb carbon dioxide nor did it liberate carbon dioxide when treated with cold dilute hydrochloric acid. The soluble material (no. 5), however, absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. These observations indicated that the substances insoluble in water which are responsible for these results are carbonates.

A graphical summary is here given of the distribution of the volumes of carbon dioxide combined by the leaf constituents in the different fractions obtained by extraction:

Frozen leaf 16.72 ml.—
- Water-soluble 4.51 ml.
- Water-insoluble 9.16 ml.—
  - Water-CO_2-soluble 11.24 ml.
  - Water-CO_2-insoluble 0.46 ml.

The sap expressed from frozen sunflower leaves (no. 7) was shown to contain substances which absorb carbon dioxide, and also to liberate carbon dioxide when treated with acid (this sample of sap was not expressed from leaves taken from the same lot as those used in experiments 1 to 6).

These experiments clearly demonstrate that sunflower leaves possess a carbon-dioxide-absorption system which is divided between the sap and the solid leaf material. This system provides a reservoir of carbon dioxide within the leaf which may be available for photosynthesis.

The notable differences between living and killed leaves in the amounts of reversible and irreversible carbon dioxide contained by each (table IV, nos. 1 and 2) may possibly be explained by the inequality in their rates of respiration.

Preliminary to measuring the carbon dioxide absorption the leaves were thoroughly evacuated. This removed all of the dissociable carbon dioxide. Since the living leaves were respiring rapidly, any carbon dioxide absorbents that they contained would be kept saturated. On the other hand, the frozen leaves respired relatively slowly and once these absorbents had been freed of dissociable carbon dioxide they would become saturated again only very slowly. Thus the living leaves would possess a low absorption capacity and a large quantity of irreversible carbon dioxide whereas frozen leaves would have these properties reversed.

Other experiments corroborated this conjecture. Three 10-gm. samples of sunflower leaves were killed in three different ways and the amounts of carbon dioxide that they retained were measured. Leaves killed with ether at room temperature retained 17.30 ml. of carbon dioxide; leaves killed by drying in an atmosphere of carbon dioxide retained 13.42 ml.; leaves killed by heating at the boiling point of water retained only 10.55 ml. These

results are what would be expected if the carbon dioxide of respiration were retained in the leaves by easily dissociable compounds. Leaves killed without heating or drying would retain the most carbon dioxide, while leaves heated without drying would retain the least.[2]

Although the counterpart of the experiment with frozen leaves has not been performed with leaves killed by heating, the latter absorb more carbon dioxide than similar living leaves. Heat-killed leaves retain a considerable quantity of carbon dioxide that is liberated only by treatment with acid.

ABSORPTION OF CARBON DIOXIDE BY PARTICULAR LEAF CONSTITUENTS

ABSORPTION BY WATER.—In order to determine the influence of water, measurements were made of the carbon dioxide absorption by matched samples of leaves which contained different amounts of water.

These samples were obtained in the following manner. Large leaves were halved and the halves segregated into two portions of 10.00 gm. each. Small leaves were thoroughly mixed and aliquot portions of 10.00 gm. each withdrawn. One portion was used immediately for absorption measurements whereas the other was partially dried before use.

The results of these experiments show that leaves with low water content absorb less carbon dioxide than those with normal water content, (fig. 3). The removal of water from leaves reduced their capacity to

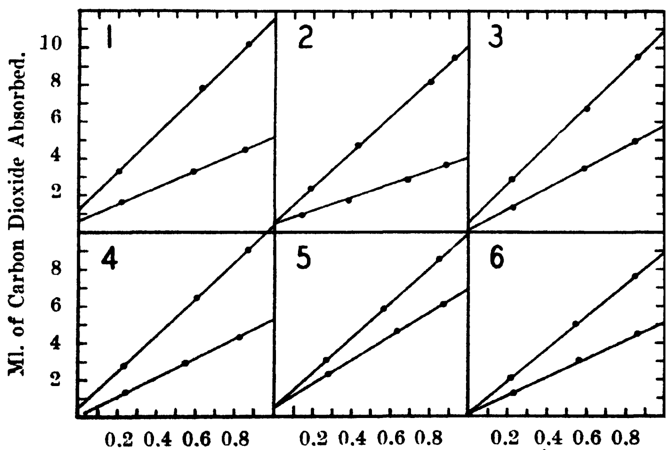


FIG. 3. The influence of water content on the absorption of carbon dioxide by leaves. The upper curves represent absorption by leaves with normal water content; the lower curves, absorption by leaves with diminished water content.

1) *Malva parviflora*; 2) *Phaseolus multiflorus*; 3) *Helianthus annuus*; 4) *Zea mays*; 5) *Hordeum vulgare*; 6) *Beta vulgaris*.

[2] The carbon dioxide retained by these various samples of leaves was estimated by liberating the carbon dioxide with cold dilute hydrochloric acid and absorbing it in a

absorb carbon dioxide to a greater extent than was calculated on the basis of water loss. This is what would be expected if the water is present in the leaf in a dilute aqueous solution such as a sugar solution. As the solution becomes more concentrated its absorption capacity is decreased both by the loss of water and by the salting-out effect (table V). Only one sample of leaves showed an apparent exception to this (table V, no. 2).

TABLE V

CHANGE IN CO_2 ABSORPTION WITH CHANGE IN WATER CONTENT. ORIGINAL FRESH WEIGHT 10.00 GRAMS: TEMPERATURE 15° C.

SAMPLE NUMBER	LEAF MATERIAL	WATER CONTENT	SOLUBILITY AT $P_{CO_2} = 1$ ATMOSPHERE	DECREASE IN WATER CONTENT	DECREASE IN CO_2 ABSORPTION	RATIO $\frac{d\,CO_2}{d\,H_2O}$
		gm.	*ml.*	*gm*	*ml.*	
1	*Zea mays* (seedlings)	8.84 3.81	10.43 5.22	5.03	5.21	1.036
2	*Hordeum vulgare*	9.06 5.83	9.98 6.92	3.23	3.06	0.947
3	*Beta vulgaris*	8.38 4.70	8.96 5.14	3.68	3.82	1.038
4	*Malva parviflora*	8.07 3.12	11.51 5.21	4.95	6.30	1.273
5	*Phaseolus multiflorus*	8.60 3.17	10.06 3.99	5.43	6.07	1.118
6	*Helianthus annuus*	8.11 3.94	10.93 5.74	4.17	5.19	1.245
7	Water					1.020
8	Sugar solution (20°)*	972.4 786.1	846.0 649.0	186.3	197.0	1.058
9	Water (20°)					0.880

* QUINN, ELTON L., and JONES, CHARLES L., Carbon dioxide, pp. 103, Reinhold Publishing Corporation. 1936.

Within the pressure range, 0.3 to 1.0 atmosphere, the solubility of carbon dioxide increased proportionally to the increase in pressure. If the increase in solubility per atmosphere of pressure is divided by the number of grams of water contained in the leaves, an apparent solubility coefficient is obtained for the water in the leaf (table VI, column 6).

The apparent solubility coefficient, in most cases, is greater than the coefficient for pure water. The coefficient increases as the water content decreases. Therefore there are substances in the leaf besides water which absorb carbon dioxide in proportion to its pressure. These substances are not lost by the partial dehydration of the leaf but increase in amount rela-

known quantity of standard barium hydroxide and determining the excess barium hydroxide with standard hydrochloric acid.

TABLE VI

CORRELATION OF THE CARBON DIOXIDE ABSORPTION COEFFICIENT OF LEAVES WITH THEIR WATER CONTENT. ORIGINAL WEIGHT OF LEAF MATERIAL, 10.00 GRAMS; TEMPERATURE 15° C.

SAMPLE NUMBER	LEAF MATERIAL	WATER CONTENT	$d\,V_{CO_2}$	dP_{CO_2}	$\dfrac{d\,V_{CO_2}}{d\,P_{CO_2}\quad W_{H_2O}}$
		gm.	*ml.*	*atm.*	
1	*Zea mays*	8.84	6.23	0.6233	1.131
		3.81	2.98	0.5842	1.339
2	*Hordeum vulgare*	9.06	5.51	0.5791	1.050
		5.83	3.77	0.5911	1.094
3	*Beta vulgaris*	8.38	5.57	0.6330	1.050
		4.70	3.13	0.6274	1.061
4	*Malva parviflora*	8.07	6.89	0.6686	1.277
		3.12	2.92	0.6354	1.473
5	*Phaseolus multiflorus*	8.60	7.05	0.7434	1.103
		3.17	2.66	0.7483	1.121
6	*Helianthus annuus*	8.11	6.65	0.6404	1.280
		3.94	3.59	0.6320	1.442
7	*Sedum praealtum*	9.26	4.79	0.5781	0.895
8	Water				1.020

tive to the water remaining. If such substances were not present the apparent solubility coefficient for carbon dioxide would be less than for pure water, due to the presence of neutral substances dissolved in the sap. In fact, leaf saps acidified in order to prevent neutralization of carbonic acid by basic constituents contained therein, possess solubility coefficients considerably less than pure water. For example, at 15° C., the solubility coefficients [ml. CO_2 (0°, 760 mm.)/gm. H_2O/atm. CO_2] for two saps acidified to pH 3.2 are: *Helianthus annuus,* 0.926; *Sedum praealtum,* 0.913. Hence it is not surprising that leaves of *Sedum praealtum* which contain an acid sap (pH 4.08) had an apparent solubility coefficient less than water (table VI, no. 7).

INFLUENCE OF HYDROGEN-ION CONCENTRATION.—Since aqueous solutions of carbon dioxide are acid, it is probable that the hydrogen-ion concentrations of leaf saps might control the ability of leaves to absorb carbon dioxide. It is obvious that in leaf saps the amount of hydroxyl ion available to react directly with carbon dioxide is too small to be of significance. The concentration of hydroxyl ion, however, may influence secondary reactions such as the formation of the alkaline-earth carbonates which are important in the process of carbon dioxide absorption. For this reason a statistical survey was made of the relation between the pH of leaf saps (obtained from heated leaves) and the amount of carbon dioxide absorbed by these leaves (corrected for the carbon dioxide dissolved by the water) at one atmosphere

pressure of carbon dioxide. The results show that little if any correlation exists between the pH of the saps and their carbon dioxide absorption (fig. 4).

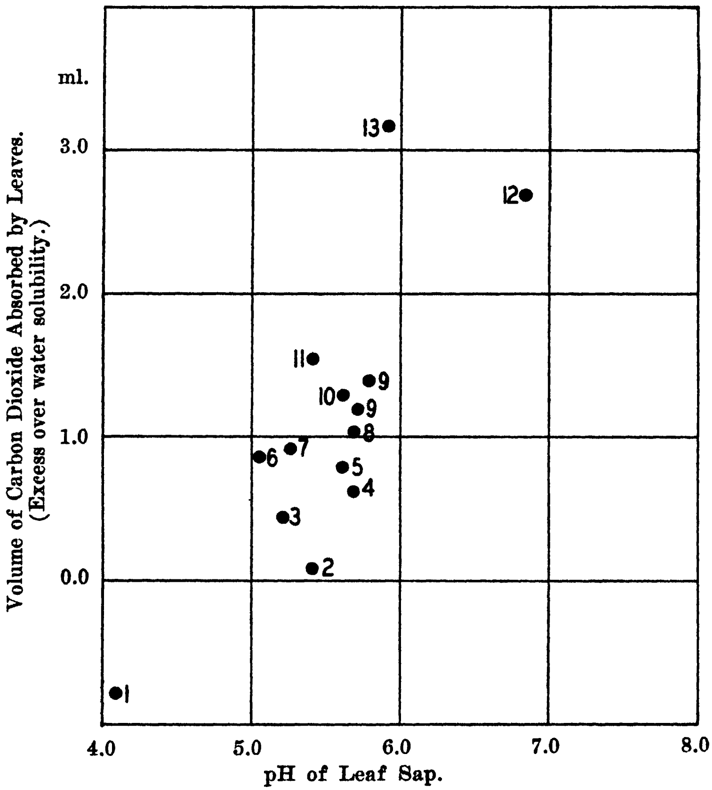


FIG. 4. Influence of hydrogen ion activity on the absorption of carbon dioxide by different leaves.

Ordinate: ml. of carbon dioxide absorbed in excess of that attributable to the water. Abscissa: pH of the saps expressed from heat-killed leaves.

1) *Sedum praealtum*; 2) *Nicotiana tabacum*, yellow with age; 3) *Beta vulgaris*; 4) *Hordeum vulgare*, etiolated; 5) *Zea mays*, etiolated; 6) *Evonymus japonicus*; 7) *Hordeum vulgare*; 8) *Nicotiana tabacum*; 9) *Zea mays*; 10) *Phaseolus multiflorus*; 11) *Evonymus japonicus*, yellow; 12) *Helianthus annuus*; 13) *Malva parviflora*.

When not otherwise specified, green leaves were used.

ABSORPTION BY LEAF-SAP CONSTITUENTS.—The absorption of carbon dioxide by the sap from frozen sunflower leaves was analyzed in order to determine what type of absorption takes place. For example, does the carbon dioxide add directly to the sap constituents, as in carbamino-acid formation, or is it neutralized to form bicarbonate ion? To differentiate between these two modes of absorption the quantity of carbon dioxide that was chemically

bound in the sap was determined and compared with the amount of bicarbonate ion formed.

PRIMARY IONIZATION CONSTANT OF CARBONIC ACID.—The most convenient way to obtain the bicarbonate ion concentration in the leaf sap is to calculate it from the equation defining the ionization constant of carbonic acid,

$$pK = pH + pHCO_3 + pf_{HCO_3} - pP_{CO_2} - pS_{CO_2},^3$$

that is, provided that the value of this constant is known and conditions of measurement can be arranged so as to warrant its use.

To establish that the experimental conditions warranted the use of this equation, the ionization constant was determined under the conditions which were used for the measurements on leaf saps. The values for the individual terms of the equation were obtained in the following ways: The activities of the hydrogen ion in the various solutions, saturated with carbon dioxide in the cell shown in figure 5, were measured by means of a Beckman pH meter.

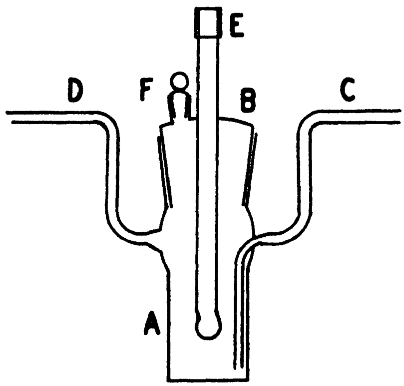


FIG. 5. Gas-reaction cell for pH measurements. A, container; B, ground-glass cap carrying the calomel electrode (not shown) and glass electrode, E; F, opening for removal of samples; C, inlet tube; D, exit for the passage of the gas stream.

The concentrations of the 0.1 M solutions of bicarbonate were determined by acidimetry.[4] The weaker solutions were made by dilution of the 0.1 M solutions. The partial pressures of the carbon dioxide were calculated from the composition of the carbon dioxide-nitrogen mixtures, the barometric pressures, and the vapor pressures of the solutions. The solubility of carbon dioxide, 0.0454 mol/liter/atmosphere, was taken from the literature (3).

[3] In this expression p is (−log of a given quantity); K, the primary ionization constant of carbonic acid; H, the hydrogen ion activity; HCO_3, the bicarbonate ion concentration; f_{HCO_3}, the activity coefficient of the bicarbonate ion; P_{CO_2}, the partial pressure of the carbon dioxide in atmospheres; S_{CO_2}, the solubility of carbon dioxide in water expressed in mol/liter/atmospheres.

[4] Merck's reagent grade of sodium bicarbonate was used for preparing the bicarbonate solutions.

The activity coefficients of the bicarbonate ion were calculated from the ionic strengths of the solutions according to the formula proposed by GUGGENHEIM and SCHINDLER (**10**).

$$-\log f = 0.5\ Z_i^2 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + \sum_k B_{ik}C_k;$$

which for the bicarbonate ion in the solutions used becomes

$$pf_{HCO_3} = 0.5 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + 0.221\ C_{Na^+} + 0.044 C_{K^+}.$$

C_{Na^+} and C_{K^+} are the molar concentrations of the sodium and potassium ions and μ is the ionic strength in the solutions measured.

In table VII are given the values of pK for the primary ionization constant of carbonic acid. The average value, pK 6.4252, is in good agreement with the value reported by SHEDLOVSKY and MACINNES (**21**), *viz.*, 6.4293 at 15.0° C.

TABLE VII

DATA FOR CALCULATING THE IONIZATION CONSTANT OF CARBONIC ACID

$NaHCO_3$ MOL/L.	$\sqrt{\mu}$	P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	pf_{HCO_3}	pK
0.0009936	0.0315	38.04	1.301	6.050	3.003	0.015	6.424
"	"	152.7	0.699	5.450	"	"	6.426
"	"	743.4	0.009	4.770	"	"	6.436
0.009936	0.0996	38.01	1.301	7.020	2.003	0.047	6.426
"	"	152.5	0.698	6.415	"	"	6.424
"	"	742.9	0.010	5.725	"	"	6.422
0.09936	0.315	37.98	1.301	7.926	1.003	0.140	6.425
"	"	152.6	0.697	7.323	"	"	6.426
"	"	742.9	0.010	6.640	"	"	6.430
					AVERAGE		6.426

IN ANOTHER SERIES OF MEASUREMENTS THE $NaHCO_3$ WAS MAINTAINED AT 0.0009936 M. AND THE IONIC STRENGTH VARIED BY ADDITION OF POTASSIUM CHLORIDE

KCL MOL/L.	$\sqrt{\mu}$	P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	pf_{HCO_3}	pK
0.01	0.1048	38.04	1.301	6.012	3.003	0.048	6.419
"	"	152.7	0.697	5.403	"	"	6.414
"	"	743.4	0.009	4.726	"	"	6.425
0.10	0.3178	38.0	1.301	5.940	"	0.125	6.424
"	"	152.6	0.697	5.330	"	"	6.418
"	"	743.4	0.009	4.656	"	"	6.432
0.25	0.501	38.05	1.300	5.888	"	0.178	6.426
"	"	152.9	0.697	5.276	"	"	6.417
"	"	744.6	0.009	4.612	"	"	6.441
					AVERAGE		6.4240
					GENERAL AVERAGE		6.4252

The average value for the change in pH with change in pP_{CO_2} was found to be 0.995 which conforms well with the theoretical value of unity. (fig. 6).

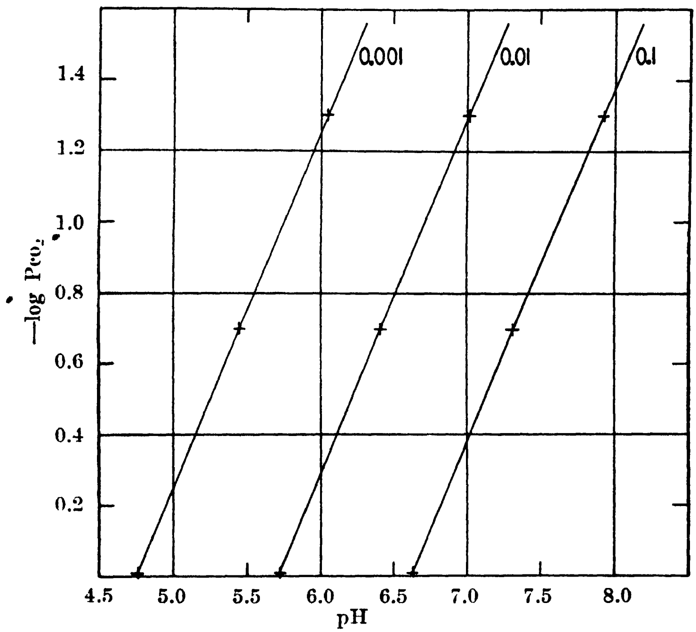


FIG. 6. The change in pH with change in $-\log P_{CO_2}$ at various bicarbonate ion concentrations (0.1, 0.01, and 0.001 M × 0.9936).

CONCENTRATION OF BICARBONATE ION IN SUNFLOWER-LEAF SAP.—The bicarbonate ion concentration of sunflower-leaf sap was calculated from the equation for the primary ionization constant of carbonic acid, pK 6.425. All other terms in the equation were measured in the manner already described with the exception of the activity coefficient of the bicarbonate ion. This was estimated by an independent experiment

For the present it is assumed that the activity coefficient of the bicarbonate ion in the sap depends chiefly upon the concentrations of the inorganic ions. To determine the concentrations of these ions, a known volume of sap was ashed and the principal inorganic ions estimated by the usual methods of analysis. The concentrations so determined, expressed in mols per liter, are shown in table VIII. The organic ions were of course destroyed by the ashing process. The solubility of calcium phosphate would be exceeded with such concentrations of calcium and phosphate ions present in the sap. This indicates that at least a part of these ions is bound in little-ionized or non-ionized forms.

By mixing the ions in the proportions obtained by analysis (supplying organic ions as acetate and formate) a "synthetic sap" was prepared which

TABLE VIII

CONCENTRATION OF IONS IN SUNFLOWER-LEAF SAP

CATION	CONCENTRATION MOLS/LITER	ANION	CONCENTRATION MOLS/LITER
Calcium	0.01306	Phosphate	0.0166
Magnesium	0.01002	Sulphate	0.0274
Potassium	0.1596	Chloride	0.0363
		Organic*	0.0815

* The organic anion concentration was calculated as the difference in the number of equivalents excess of cations over anions and includes bicarbonate ion.

Note.—Allowing for the solubility of calcium phosphate as 0.561 gm. per liter of water saturated with carbon dioxide (20) the ionic strength of the sap was calculated as 0.2434. Applying the formula of GUGGENHEIM and SCHINDLER (10) and calculating the specific effect of the cations to be the same as an equal concentration of potassium ions, a value of $pf_{HCO_3} = 0.173$ was obtained.

was free from any compound that might bind carbon dioxide in any way except by neutralization. When the components were mixed, a precipitate appeared which was dissolved by bubbling carbon dioxide through the solution. On standing over night, calcium phosphate crystallized out of the solution. After standing at room temperature for several days the crystals were removed by filtration. The supernatant liquid was saturated with carbon dioxide at known partial pressures in the gas reaction cell (fig. 5) and the activity of the bicarbonate ion determined. The value of the activity coefficient found was $f_{HCO_3} = 0.665$ ($pf_{HCO_3} = 0.177$). A closely-agreeing value $f_{HCO_3} = 0.662$ ($pf_{HCO_3} = 0.179$) was obtained also by the use of a similar solution containing glucose (approx. 0.176 M).

The bicarbonate concentrations used in calculating the activity coefficients of bicarbonate ions were determined by means of the VAN SLYKE and NEILL blood-gas analysis apparatus (**31**). Since the solutions analyzed were saturated with carbon dioxide it was necessary to subtract the amount of dissolved carbon dioxide in order to obtain the bicarbonate concentration. This solubility was estimated by an independent measurement of the solubility of carbon dioxide in the solution. For this determination the solution was slightly acidified (pH 3.47) with concentrated hydrochloric acid.

An independent estimate of the activity coefficient of the bicarbonate ion was made from the ionic strength of the solution. The value calculated, $f_{HCO_3} = 0.671$ ($pf_{HCO_3} = 0.173$), corroborated the value obtained by direct experiment.

The following equation was obtained by substituting these experimentally determined values:

$$p_{HCO_3} = 6.425 + 1.343 - 0.177 + pP_{CO_2} - pH$$

This reduces to the simplified expression

$$p_{HCO_3} = 7.591 + pP_{CO_2} - pH$$

In table IX and figure 7 the concentrations of bicarbonate ion calculated from this equation are compared with the total amount of bicarbonate bound

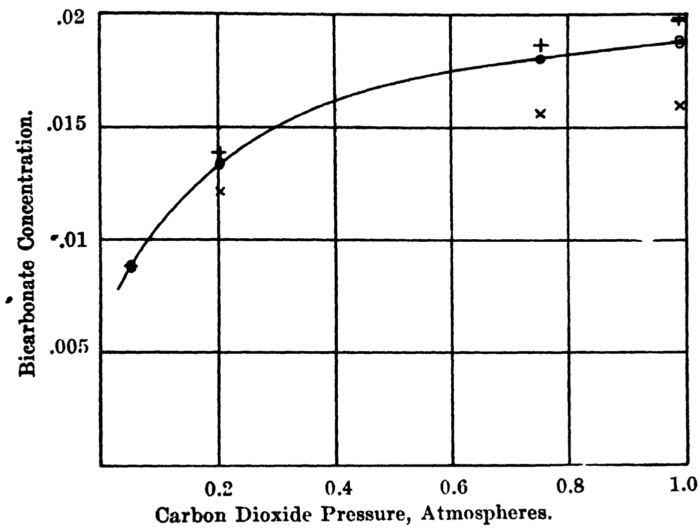


FIG. 7. The bicarbonate ion concentration determined from electromotive force measurements (+) compared with the total combined carbon dioxide obtained by gas-analytical methods (O) and that calculated from the buffer action of the phosphates (X) in sunflower-leaf sap.

TABLE IX

BICARBONATE-ION CONCENTRATION OF SUNFLOWER LEAF SAP

P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	HCO_3 (E.M.F.)	HCO_3 (ANAL.)
atm.	*atm.*		*mol/l.*	*mol/l.*	*mol/l.*
0.05065	1.295	6.831	2.055	0.00881	0.00875
0.05063	1.296	6.831	2.056	0.00879	0.00886
0.2030	0.693	6.424	1.860	0.01380	0.01326
0.2030	0.693	6.424	1.860	0.01380	0.01339
0.7536	0.123	5.984	1.730	0.01863	0.01801
0.7533	0.123	5.984	1.730	0.01863	0.01802
0.9881	0.005	5.891	1.705	0.01973	0.01867
0.9882	0.005	5.892	1.704	0.01977	0.01884

chemically by the sap as determined by analytical means (19). This comparison shows that the two sets of values are nearly equal. The conclusion may be drawn, therefore, that all of the combined carbon dioxide in the leaf sap was present as bicarbonate ion and that none of it had added directly to the sap constituents to form carbamino-like compounds.

THE CARBON DIOXIDE ABSORBENTS IN SUNFLOWER-LEAF SAP.—From the foregoing experiments it is clear that, in sunflower-leaf sap, buffer sub-

stances are present which are capable of reacting with carbon dioxide; otherwise the bicarbonate ion concentration would not increase with increase in pressure of carbon dioxide.

By correlating the phosphate content with the neutralization capacity of the sap from the hypocotyl of sunflower plants, MARTIN (15) concluded that the buffer action was due almost entirely to phosphates. From the experiments on sunflower leaves reported here it is apparent that the increase in bicarbonate ion concentration with increase in carbon dioxide pressure is too large to be accounted for solely by the phosphates (table X, fig. 7). Also

TABLE X

CHANGE IN SECONDARY PHOSPHATE CONCENTRATION IN SUNFLOWER-LEAF SAP WITH CHANGE IN PRESSURE OF CARBON DIOXIDE*

pH‡	$-\log \frac{HPO_4^=}{H_2PO_4^-}$	$\frac{HPO_4^=}{H_2PO_4^-}$	$HPO_4^=$	P_{CO_2}	HCO_3† (CALC.)	HCO_3† (OBS.)
6.831	−0.115	1.303	0.009390	0.0507	(0.00875)	0.00875
6.424	0.292	0.5105	0.005611	0.2030	0.01213	0.01326
5.984	0.732	0.1854	0.002596	0.7536	0.01554	0.01801
5.891	0.825	0.1496	0.002160	0.9881	0.01598	0.01867

* In these calculations the value of $\mu = 0.2434$, was taken as the ionic strength of the sap.

The calculations were then made from the formula

$$-\log \frac{HPO_4^-}{H_2PO_4^-} = 7.211 - 0.495 - pH$$

† HCO_3 calculated is the sum of the bicabonate ion concentration determined analytically at 0.05 atmospheres carbon dioxide plus the increase calculated from the change in primary phosphate ion.

HCO_3 observed was determined by means of the blood gas analysis apparatus.

‡ The pH measurements in this paper are referred to the pH values for standard buffers given by MACINNES, BELCHER, and SHEDLOVSKY (14): for the standard 0.1 N acetate buffer pH 4.650, and for the potassium acid phthalate, 0.05 M, pH 4.000.

the neutralization of the sap by acid (fig. 8) demonstrates that the sap contains other neutralizing substances. While phosphates may be the chief buffers, other buffer substances are present which have not yet been identified.

From separate experiments the second ionization constant of phosphoric acid, on which the calculations for table X and figure 7 are based, was found to be defined by the following equation:

$$pK = pH - \log \frac{HPO_4^=}{H_2PO_4^-} + \frac{1.5\sqrt{\mu}}{1+\sqrt{\mu}} \qquad (9).$$

The value estimated for pK is 7.211 at 15.0° C. (table XI).[5]

By determining the pH and applying this equation, the change in the

[5] A value pK = 7.228 (15.0°) was estimated from the values given by NIMS (16) extrapolated to 15.0° by the formula of HARNED and EMBREE (11).

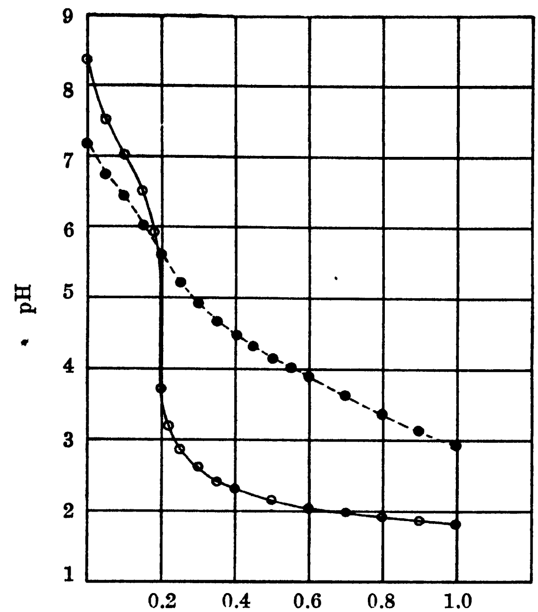


FIG. 8. Electrometric titration curves of sunflower-leaf sap (●) and of 0.02 M dipotassium phosphate (O). Ordinate, pH; abscissa, volume of N/1 × 0.9685 hydrochloric acid. (10.00 ml. of each solution were used.)

ratio of secondary to primary phosphate was calculated. From this ratio and the total phosphate content of the sap the increase in bicarbonate ion concentration was determined. This was found to be considerably less than the increase in bicarbonate ion concentration determined analytically (table X).

The method of estimating bicarbonate ion concentration, from the trans-

TABLE XI

DETERMINATION OF THE IONIZATION CONSTANT OF THE DIHYDROGEN PHOSPHATE ION. THE EFFECT OF POTASSIUM CHLORIDE. RATIO OF SECONDARY TO PRIMARY PHOSPHATE = 1.0; TOTAL CONCENTRATION OF PHOSPHATE ION = 0.01 M; TEMPERATURE, 15.0° C.

CONC. KCl MOL/L.	μ KCL	μ PO$_4$	μ TOTAL	$\frac{1.5\sqrt{\mu}}{1.0+\sqrt{\mu}}$	pH	pK
0.0	0.0	0.02	0.020	0.185	7.02_5	7.210
0.10	0.10	0.02	0.120	0.386	6.82	7.206
0.20	0.20	0.02	0.220	0.478	6.73_3	7.211
0.50	0.50	0.02	0.520	0.628	6.58_5	7.213
0.85	0.85	0.02	0.870	0.723	6.49	7.213
					AVERAGE	7.211

formation of secondary into primary phosphate ion, was verified by a separate experiment. A solution of dipotassium hydrogenphosphate (0.0184 M) was saturated with carbon dioxide. From the pH (5.908) of the resultant solution the change in concentration of secondary phosphate was calculated. The amount of secondary phosphate transformed was found to be equal to the increase in bicarbonate ion determined either by the analytical method of VAN SLYKE and NEILL (31) or by the ionization constant equation for carbonic acid. A comparison of the values obtained by these independent methods is here tabulated:

Method used	Equation $K_{H_2PO_4^-}$	Equation $K_{H_2CO_3}$	Gas-analytical
Bicarbonate concentration obtained	0.01693	0.01648	0.01635

These results justified the methods of calculation used for the estimation of bicarbonate ion concentration in sunflower-leaf sap.

In summarizing, it may be said that phosphates probably play an important rôle in the buffer action of sunflower-leaf sap toward carbonic acid; other substances are present, however, in the sap which react with carbonic acid. This was demonstrated by obtaining the neutralization curve of sunflower-leaf sap and also by calculating the amount of carbonic acid that could be neutralized by the quantity of secondary phosphate transformed.

ABSORPTION OF CARBON DIOXIDE BY THE WATER-INSOLUBLE LEAF RESIDUE

The killed leaf, even after extraction with water, absorbed carbon dioxide. The ash of this solid leaf residue contained calcium and magnesium salts, the amounts of which accounted completely for the alkalinity of the ash (table XII). Inasmuch as the extracted leaf residue liberated carbon

TABLE XII

BASICITY OF THE ASH FROM THE INSOLUBLE SUNFLOWER-LEAF RESIDUE

Gram atoms of calcium	6.69×10^{-4}
Gram atoms of magnesium	1.80×10^{-4}
Sum	8.49×10^{-4}
Equivalents of base	16.98×10^{-4}
Equivalents of acid used for neutralization	16.57×10^{-4}

dioxide when treated with cold dilute hydrochloric acid, the active absorptive agents in the residue were probably calcium and magnesium carbonates. If such were the case, an extraction of the leaf residue with water saturated with carbon dioxide would remove these salts. This would decrease the calcium and magnesium content of the residue and at the same time remove its ability both to absorb carbon dioxide and also to liberate carbon dioxide

on treatment with acid. Furthermore, alkaline-earth carbonates would be found in the extract. This prediction was verified as shown in table XIII.

TABLE XIII

RELATION OF CARBON DIOXIDE ABSORPTION TO ALKALINE EARTH CARBONATE CONTENT. QUANTITIES BASED ON 10.00 GRAMS OF FRESH LEAVES. ALL QUANTITIES ARE EXPRESSED IN MOLS × 10^4

SAMPLE NUMBER		FRESH LEAF	FROZEN LEAF	RESIDUE FROM H_2O EXTRACTION	WATER EXTRACT	RESIDUE FROM H_2O–CO_2 EXTRACTION	H_2O–CO_2 EXTRACT
1	Reversible CO_2	1.34	3.99	2.19	2.17	0.20	2.67
2	Irreversible CO_2	7.09	3.52	1.92	− 0.17	0.00	2.38
3	Combined CO_2	8.43	7.51	4.12	2.00	0.20	5.05
4	Calcium	5.59	6.18	6.48	0.78	3.29	2.92
5	Magnesium	2.38	2.46	1.69	0.87	0.49	1.03
6	Calcium + magnesium	7.97	8.64	8.17	1.65	3.78	3.95

These data make it apparent that a close correlation exists between the content of alkaline earths and the amount of carbon dioxide that can be combined by the leaf residue.

In order to confirm the observation that water saturated with carbon dioxide removed calcium and magnesium carbonates from water-insoluble leaf residue, the dissolved material was isolated in solid form.

Frozen sunflower-leaf material (210 gm.) was prepared as has already been described. Water-soluble material was removed by two extractions with water (2500 ml. each). The insoluble residue was then extracted twice with water saturated with carbon dioxide (2500 ml. each). The carbonic acid extracts were concentrated to 1500 ml. by boiling and the solid which precipitated was filtered off. This solid was extracted with two portions of water saturated with carbon dioxide (500 ml. each). By boiling this extract a solid was again precipitated which was collected and dried. It weighed 0.2459 gm.

Analysis showed the precipitate to have the following composition:

Constituent	Per cent.	Constituent	Per cent.
CaO	51.45	CO_2	33.39
MgO	1.29	Volatile not CO_2	3.47
MnO	0.65		
P_2O_5	10.86	Total	101.11

This solid material absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. 0.0257 gm. of this material liberated 4.34 ml. of carbon dioxide when treated with acid. The same quantity of solid absorbed 1.83 and 2.40 ml. of carbon dioxide at 0.678 and 0.891 atmospheres pressures of carbon dioxide, respectively (volumes reduced to 0°, 760 mm.).

It has been demonstrated by analytical and preparative procedures that the absorptive agents in the water-insoluble residue from sunflower leaves are calcium and magnesium carbonates and perhaps phosphates. The presence of manganese in the carbonate precipitates is noteworthy in view of the recent experiments which show that manganese may play a rôle in the absorption of carbon dioxide by water plants (**2**) and, under certain conditions, may stimulate photosynthesis (**17**).

Alkalinity of saps from leaves treated with carbon dioxide

Leaves from some plants when placed in an atmosphere containing high concentrations of carbon dioxide yield an expressed sap which is more alkaline than the sap from leaves maintained under normal conditions (**30**). Fife and Frampton (**8**) investigated this phenomenon under a variety of conditions and concluded that under the influence of high concentrations of carbon dioxide the plants catalyzed the conversion of acid amides into ammonium salts and on removal of the carbon dioxide the plants catalyzed the reverse reaction.

$$2\ RCONH_2 + 3H_2O \underset{(-CO_2)}{\overset{(+CO_2)}{\rightleftharpoons}} RCOONH_4 \quad RCOOH + NH_4^+ + OH^-$$

This hypothetical mechanism was supported by the facts that the quantity of ammonia nitrogen increased and amide nitrogen decreased when the leaves were placed in an atmosphere of high carbon dioxide content, whereas the reverse occurred when the leaves were transferred to ordinary air.

In view of the results reported in the preceding section it seemed probable that the formation of alkaline-earth bicarbonates might also be involved in this phenomenon.

Water charged with carbon dioxide removes calcium and magnesium carbonates from the water-insoluble sunflower-leaf residue. When the leaf is stored in high concentratitons of carbon dioxide gas carbonic acid concentration of the sap will increase and dissolve additional amounts of the alkaline-earth carbonates. The soluble calcium and magnesium bicarbonates so formed will be expressed with the sap and increase the pH of the sap by repressing the acidity of the carbonic acid according to the equation:

$$[H^+] = \frac{K[H_2CO_3]}{[HCO_3^-]}.$$

To test this hypothesis three lots of sunflower leaves were frozen

(– 70° C.) in three different atmospheres: one lot in air; one in nitrogen; and one in carbon dioxide. The sap from each lot was pressed out and the pH values of the sap measured under the following conditions: just as the sap came from the press; after being swept with nitrogen gas; after being saturated with carbon dioxide gas. The data from these experiments are recorded in table XIV.

TABLE XIV

ALKALIZATION OF SUNFLOWER LEAF SAP

	Leaves treated with		
	Nitrogen	Air	Carbon dioxide
Original pH of sap	6.82	6.82	7.02
Saturated with carbon dioxide	6.06	6.03	6.38
Sap flushed with nitrogen	7.07	7.13	8.22
Molarity in sap:			
Calcium	0.0204	0.0206	0.0250
Magnesium	0.0211	0.0216	0.0230

The sap expressed from the carbon dioxide-treated leaves is less acid than the saps from leaves treated with either air or nitrogen. This result confirms the observations of FIFE and FRAMPTON. The sap expressed from leaves treated with carbon dioxide becomes less acid when saturated with carbon dioxide gas than do the saps from leaves treated with air and with nitrogen. When flushed with nitrogen the sap from carbon dioxide-treated leaves becomes more alkaline than the saps from the nitrogen or air-treated leaves. Treatment of leaves with carbon dioxide increases the calcium and magnesium content of the sap.

These observations can be correlated by assuming that alkaline earth carbonates are removed from the structural material of the leaf and dissolved in the leaf sap as bicarbonates by treatment with carbon dioxide. Undoubtedly this reaction also is involved in the alkalization of sunflower-leaf sap by treatment of the leaf with carbon dioxide.

What relation the absorption of carbon dioxide by the alkaline-earth carbonates bears to photosynthesis in land plants is not known. If, however, land plants can utilize carbon dioxide bound to the alkaline earths as well as water plants do, this mechanism of carbon dioxide absorption may be of considerable significance to them (1).

ABSORPTION OF CARBON DIOXIDE BY CHLOROPHYLL

One of the intriguing questions concerning photosynthesis is how the energy absorbed by chlorophyll is used to reduce carbon dioxide. Chemical

combination between chlorophyll and carbon dioxide has been one answer. It is of interest, therefore, to determine whether any evidence exists from previous work for such a combination. Two methods have been used to obtain such evidence. One method has been to determine whether chlorophyllous tissues absorbed more carbon dioxide than did non-chlorophyllous tissues; the other method has been to find out whether isolated chlorophyll and its derivatives exhibited any tendency to react with carbon dioxide. The results obtained previously have not been conclusive and in some cases have been actually contradictory. Because of the importance of the conception of a pigment-carbon-dioxide complex to the formulation of a proper scheme for the mechanism of photosynthesis, further evidence regarding the existence of such a complex has been sought.

In regard to the absorption of carbon dioxide by green and yellow varieties of leaves WILLSTÄTTER and STOLL (33b) obtained no difference between such varieties of elder and elm. On the other hand SPOEHR and McGEE

TABLE XV

CARBON DIOXIDE ABSORPTION* BY CHLOROPHYLLOUS AND NON-CHLOROPHYLLOUS LEAVES

No.	TYPE OF VARIANT	SPECIES	CHLOROPHYLLOUS	NON-CHLOROPHYLLOUS	CO_2-EQUIV. CHLOROPHYLL (GREEN VARIETIES)	pH OF CHLOROPHYLLOUS	pH OF NON-CHLOROPHYLLOUS
			ml.	*ml.*	*ml.*		
1	Etiolated	*Nicotiana tabacum*	1.02	0.34			
2	"	*Hordeum vulgare*	1.09	0.65	0.38	5.78	5.77
3	"	"	0.70	0.75	0.39	5.78	5.78
4	"	*Phaseolus multiflorus*	1.30	2.81			
	"	*Zea mays*	1.19	0.85	0.64	5.72	5.62
6	"	*Pisum sativum*	1.44	0.85	0.50	5.92	5.62
7	Aureous variety	*Evonymus japonicus*	0.92	1.52	0.41	5.05	5.42
8	Albino	*Zea mays*	1.41	1.55		5.78	5.75
9	"	" "	1.02	1.37	0.36	5.52	5.68
10	Yellow with age	*Nicotiana tabacum*	1.02	0.03	0.39	5.68	5.41

* The absorption values are given as cubic milliliters of CO_2, (0°, 760 mm.) absorbed at one atmosphere of CO_2-pressure by 10.00 grams of fresh leaf material, in excess of that ascribable to water. The CO_2-equivalent of chlorophyll was based on the ratio of one mol of carbon dioxide to one mol of pigment. Measurements were made by the use of apparatus shown in figure 1.

(25) found indication that "green leaves and stems absorb considerably more carbon dioxide than the corresponding etiolated portions."

The effect of chlorophyll on the absorption of carbon dioxide by leaves has been re-examined. It has been found that sometimes chlorophyllous and sometimes non-chlorophyllous leaves absorb the more carbon dioxide. No consistency has been obtained (table XV).

In order to gain a more sound basis of comparison between chlorophyllous and non-chlorophyllous leaf material, the absorption of carbon dioxide by the white and by the green portions of the same variegated ivy leaves was measured. The colorless portions absorbed 1.3 ml. and the green parts 0.94 ml. of carbon dioxide per unit weight at 0.25 atmospheres of pressure, in excess of that ascribable to the water present. This experiment confirmed the results obtained by the use of other non-chlorophyllous materials.

Further evidence regarding the effect of chlorophyll was obtained in the following way. 10.00 gm. of fresh leaves were frozen, thawed, and extracted with water charged with carbon dioxide. This removed all water-soluble carbon dioxide absorbents, but left the chlorophyll in the leaf residue intact. This chlorophyll-containing residue absorbed no more carbon dioxide (0.21 ml.) than did a similar residue from which the chlorophyll had been extracted with alcohol (0.27 ml.). The amount absorbed in both cases was insignificant. No carbon dioxide was liberated with cold dilute acid from either sample.

From these experiments it may be concluded that chlorophyll is not the factor controlling the absorption of carbon dioxide by leaves and no evidence has been obtained that the chlorophyll in leaves unites with carbon dioxide.

Previous experiments with isolated chlorophyll had indicated that chlorophyll united with carbon dioxide. The experiments of WILLSTÄTTER and STOLL demonstrated that colloidal chlorophyll in water suspension absorbs carbon dioxide (33d). Most of the absorbed carbon dioxide could be accounted for by the reaction with, and removal of, the magnesium in the pigment. There was, however, an additional absorption which was attributed to the pigment itself.

A reinvestigation of this reaction (carried out at 25.1° instead of 0°, the temperature used by WILLSTÄTTER and STOLL) confirmed the observation that carbon dioxide is absorbed and magnesium removed from the pigment. The amount of carbon dioxide absorbed in excess of that calculated for the water was less than the amount necessary to form magnesium bicarbonate with the magnesium removed from the pigment.

From the reaction rate curve (fig. 9) it is apparent that the reaction had not reached completion. The rate of absorption had become so slow, because of the flocculation of the pigment, that it seemed unprofitable to con-

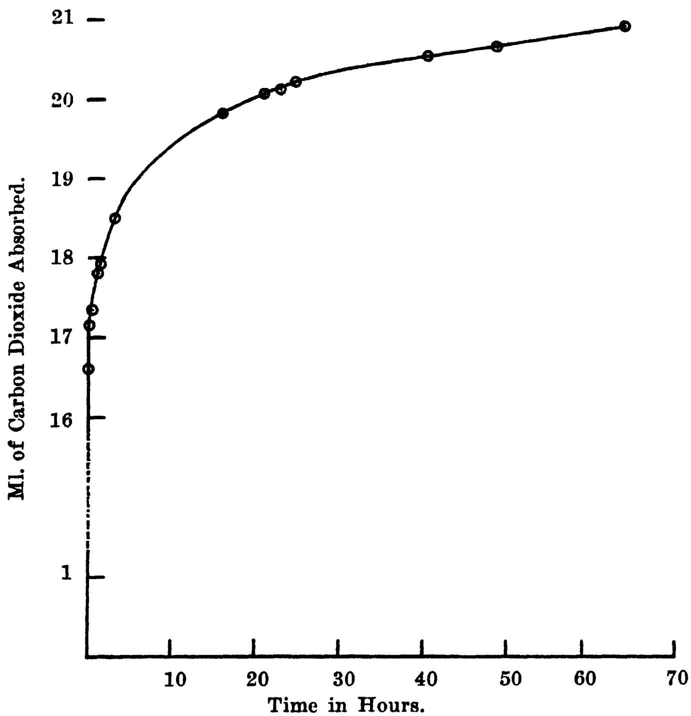


FIG. 9. Curve representing the rate of absorption of carbon dioxide by an aqueous suspension of colloidal chlorophyll.

tinue the experiment longer. A summary of the results is given in table XVI.

TABLE XVI

ON THE ABSORPTION OF CARBON DIOXIDE BY COLLOIDAL CHLOROPHYLL AND THE SPLITTING OUT OF THE MAGNESIUM BY THE CARBON DIOXIDE

Volume of solution of colloidal chlorophyll (ml.)	20.45
Weight of colloidal chlorophyll (gm.)	0.32
Volume of chlorophyll (ml.)	0.29
Volume of water (ml.)	20.13
Pressure of CO_2 (mm.)	737.2
Temperature (0° C.)	25.1
Vol. CO_2 (0°, 760 mm.) required by water (ml.)	14.82
Vol. CO_2 dissolved by suspension (ml.)	20.85
Vol. of CO_2 reduced to standard conditions (ml.)	18.52
Excess vol. dissolved by suspension (ml.)	3.70
Weight of CO_2 dissolved (mg.)	7.31
Mols of CO_2 dissolved $\times 10^4$	1.661
Atoms of magnesium removed $\times 10^4$	1.114
Ratio CO_2/Mg	1.491

The chlorophyll used in this experiment was obtained by the method of

WILLSTÄTTER and STOLL (32a). The colloidal suspension was prepared by HUBERT's method (12), 0.5 gm. of chlorophyll being dispersed in 32 ml. of colloidal suspension. HUBERT's method was modified only in that the acetone solution of chlorophyll was forced into the rapidly stirred water by gravity rather than by compressed air.

The absorption of carbon dioxide by the colloidal suspension of chlorophyll was measured in the apparatus diagrammed in fig. 10. The apparatus

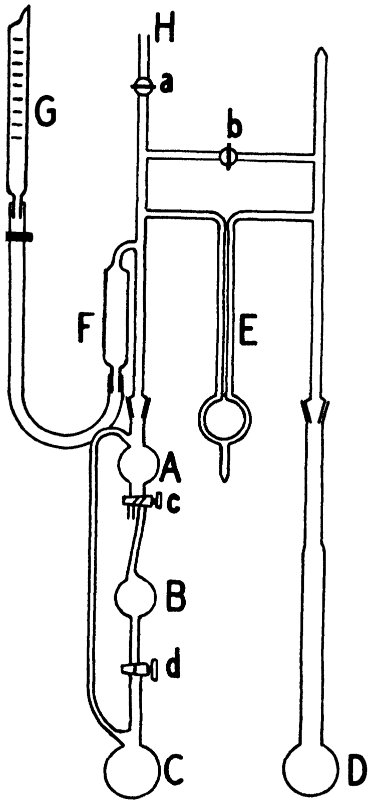


FIG. 10. Apparatus for measuring the solubility of carbon dioxide in a colloidal suspension of chlorophyll in water.

was patterned after the one previously used in this laboratory for microhydrogenation (22).

The suspension was introduced into bulb A. With stopcocks *a*, *b*, and *d* open and *c* closed, the air was removed from the apparatus and the dissolved gases pumped out of the colloidal suspension. Stopcock *d* was then closed and *c* opened so that the suspension ran into the calibrated pipet B. Stopcock *a* was opened and air allowed to enter. The apparatus was again

evacuated and any bubbles that formed in B removed. This alternate evacuation and filling with air was continued until pipet B was completely full of liquid. Stopcock *c* was then turned so as to drain the excess solution out of bulb A. When all had drained out, *c* was closed. The apparatus was then alternately evacuated and filled with carbon dioxide until it contained pure carbon dioxide. The whole apparatus was lowered into the constant temperature bath (25.1° C.) and shaken until the water contained in D and the rest of the apparatus was saturated with carbon dioxide. Stopcock *b* was closed and as soon as the manometer liquid in the two arms of the manometer E had remained level for a half-hour the solution was allowed to flow into the reaction bulb C by opening stopcocks *c* and *d*. Absorption was evidenced by the change in level of the manometer liquid. To equalize the levels of the liquid in the two arms of the manometer, mercury was let into the reservoir F from buret G. When equilibrium was established, as shown by the constancy of the manometer, the volume of the gas absorbed was read from the buret.

The auxiliary apparatus connected to the absorption apparatus at H is not pictured. It consisted of a carbon dioxide generator, washflasks, manometer, and pumps so arranged with stopcocks that the evacuation and filling of the absorption apparatus with the desired gases could be effected readily.

This absorption apparatus gave the accepted value for the Bunsen absorption coefficient of carbon dioxide in water, 0.753 at 25.1° C. BOHR found the value, 0.757 (3).

The experimental data for the absorption of carbon dioxide by the colloidal suspension of chlorophyll are given in table XVI and figure 9.

During the period of absorption, 64 hours, the colloidal chlorophyll flocculated and precipitated out. As soon as the experiment was completed the reaction vessel was removed from the apparatus, the solution filtered from the separated chlorophyll, and the magnesium in the filtrate estimated as the 8-hydroxyquinolate; the precipitated magnesium complex was determined gravimetrically. The number of atoms of magnesium recovered was 1.114×10^{-4}. The number of mols of chlorophyll used was 3.54×10^{-4}. Therefore, not all of the magnesium had been removed from the chlorophyll.

Recalculation of the results of WILLSTÄTTER and STOLL (33g) showed that the solubility of carbon dioxide in suspensions of colloidal chlorophyll exceeded that necessary to saturate the water and to form magnesium bicarbonate by about 0.1 mol of carbon dioxide per mol of pigment. The solubility of carbon dioxide in the solid pigments approaches this value. This suggests that the results of WILLSTÄTTER and STOLL may be explained by the solubility of the carbon dioxide in the solid pigment phase in the colloidal suspension, in which case no evidence remains for the chemical union between chlorophyll and carbon dioxide.

RABINOWITCH (18) has recently reported that solid ethylchlorophyllide absorbs carbon dioxide. The extrapolated limit of absorption is two mols of carbon dioxide to one of pigment. This value is suggestive of compound formation between chlorophyll and carbon dioxide.

To ascertain whether evidence also exists for compound formation between chlorophyll and carbon dioxide, the solubility of carbon dioxide in solid chlorophyll (a + b) and in pheophytin (a + b) was measured. Within the limits of accuracy of the apparatus the absorption appeared to be directly proportional to the pressure of the carbon dioxide (fig. 11). This

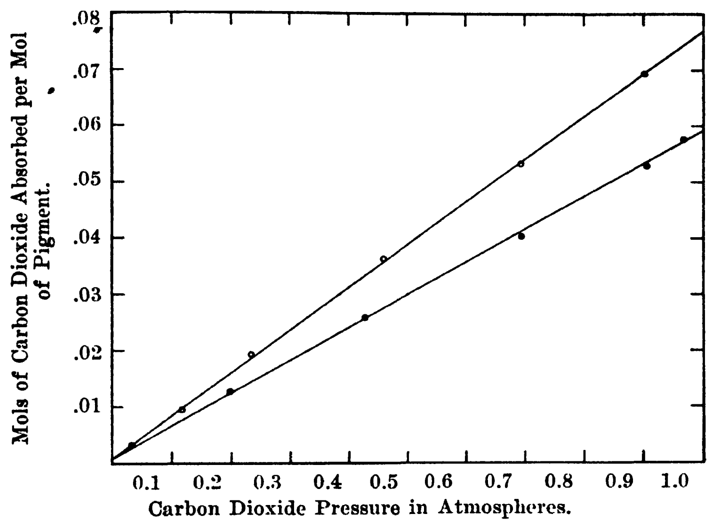


FIG. 11. The absorption of carbon dioxide by chlorophyll and pheophytin in the solid state. Temperature 0.1° C.

Ordinate: mols of carbon dioxide absorbed per mol of pigment. Abscissa: pressure of carbon dioxide in atmospheres. Chlorophyll ●; pheophytin O.

pointed to physical solution rather than to chemical combination of the carbon dioxide. When pheophytin was not thoroughly dry a slight deviation from linearity was obtained which may have been the result of chemical action.

The absorption of carbon dioxide by solid chlorophyll was carried out at 0.1° C. in the apparatus shown in figure 1. The chlorophyll had been prepared from mallow leaves by the method of WILLSTÄTTER and STOLL (32d). It contained 2.68 per cent. magnesium.

The method of measurement was the same as that which has already been described except that the free volume of the reaction vessel (49.54 ml.) was calculated from the known volume of the vessel and the volume of the chlorophyll. [The weight used was 3.2022 gm. and the density taken was 1.11, the value given by KETELAAR and HANSON (13)].

Determination of the free volume of the reaction vessel by admission of nitrogen and hydrogen gave values which were larger than that calculated from the geometry of the system (nitrogen, 49.66; hydrogen, 49.72 ml.). On the assumption that the larger values were caused by the solubility of these gases in the pigment, solubility coefficients of nitrogen and hydrogen in chlorophyll were calculated. A comparison of the solubilities of nitrogen, hydrogen, and carbon dioxide in chlorophyll at 0.1° C. and 760 mm. pressure are tabulated:

Gas	Solubility ml.
Nitrogen	0.0387
Hydrogen	0.0547
Carbon dioxide	1.472

The solubilities are expressed as cubic milliliters of gas absorbed (reduced to standard conditions) by one gram of pigment per atmosphere of pressure of carbon dioxide.

The absorption of carbon dioxide by chlorophyll was very rapid. Within five minutes after admission of the gas, equilibrium was established. The absorption was completely reversible: the volume absorbed was 4.57 ml. (manometrically); the volume removed by pumping was 4.59 ml. (volumetrically).

The absorption of carbon dioxide by pheophytin was measured in the same way as described for chlorophyll. The solubility of carbon dioxide in pheophytin was found to be 1.963 ml. per gm. of pigment per atmosphere of carbon dioxide pressure.

Pheophytin was prepared from the chlorophyll used in the previous experiments. The chlorophyll was dissolved in 200 ml. of 95 per cent. ethanol. To this solution 12 ml. of water and 1 ml. of concentrated hydrochloric acid were added and the mixture shaken for thirty minutes. The pheophytin which precipitated was filtered off and washed with distilled water until the washing no longer reacted acid to litmus. The pigment was dried over calcium chloride, then in an Abderhalden pistol at the boiling point of methyl alcohol and finally stored over calcium chloride in a vacuum desiccator for two days. This material contained 0.22 per cent. ash.

According to WILLSTÄTTER and STOLL (32b), pheophytin is not extracted from ether solution with hydrochloric acid less concentrated than 25 per cent. and is almost completely extracted by 32 per cent. Only a trace of this pigment was removed from ether solution by 22 per cent. hydrochloric acid and a large proportion was removed by 37 per cent. acid. From these tests it was concluded that the pigment still contained the phytyl group.

During its transfer to the reaction vessel, the pigment appeared to take up moisture. To remove this moisture the reaction vessel containing the pigment was warmed (40° to 45° C.) and alternately pumped out with the Sprengel pump and flushed with dry nitrogen. The sweeping process was continued until the volume of the nitrogen admitted to the reaction vessel (measured manometrically) was the same as the volume recovered by pumping.

The absorption was directly proportional to the pressure of the carbon dioxide (fig. 11).

Liberation of carbon dioxide with boiling hydrochloric acid

One question concerning the absorption of carbon dioxide by unilluminated leaves is whether other compounds besides carbonates and bicarbonates exist in leaves which can make carbon dioxide easily available to the leaf. An attempt was made to answer this question by determining the amounts of carbon dioxide liberated from leaves by boiling the leaves with hydrochloric acid of different concentrations.

Tobacco leaves, which had been stored in the dark for four days, were used in these experiments. Samples (25 gm.) of the parenchymatous tissues were cut from the mid-rids and put into a flask containing 125 ml. of the acid solution to be tested. The leaf material was boiled for one hour and the liberated carbon dioxide measured.

Experiments were performed with solutions containing 0, 1, 5, and 12 per cent. hydrochloric acid, respectively. The amounts of carbon dioxide liberated were:

Hydrochloric acid used, per cent.	0	1	5	12
Carbon dioxide liberated, mg.	6.62	9.95	16.18	51.70

The periods of boiling were probably too short to produce the maximum quantities of carbon dioxide, but it is evident that carbon dioxide may be liberated from leaf material with different degrees of facility. There was some evidence that storage of the leaves depleted the amount of carbon dioxide that could be liberated with 12 per cent. hydrochloric acid.

Summary

Measurements of the carbon dioxide absorption by unilluminated leaves have demonstrated that all leaves so far examined, with the possible exception of leaves from the acid plant *Sedum praealtum,* absorb carbon dioxide in excess of that ascribable to the water they contain. Inasmuch as etiolated, yellow, and albino leaves exhibit as great an absorption as their chlorophyllous counterparts, it appears that chlorophyll is not the controlling factor in carbon dioxide absorption. This is confirmed by the fact that

the extracted leaf residues which still contain chlorophyll give no evidence of compound formation with carbon dioxide. Furthermore, chlorophyll and its magnesium-free derivative, pheophytin, both absorb carbon dioxide but show no indication of combining with it chemically.

By comparing the amount of carbon dioxide absorbed with that removed from the leaf by evacuation, it has been demonstrated that the absorption process is strictly reversible. This is true for killed as well as for living leaves.

The water in leaves absorbs carbon dioxide in proportion to the quantity of water present and in proportion to the partial pressure of the carbon dioxide.

Detailed analysis of absorption by sunflower leaves revealed that both the sap and the insoluble leaf residue absorb carbon dioxide. The absorption in the sap may be accounted for by the reaction of carbonic acid with the buffers present, chiefly with the phosphates. The absorption by the insoluble material is attributable to the alkaline-earth carbonates contained therein.

The author wishes to thank Professor A. C. Frazer of Cornell University for furnishing the seed from which the albino corn plants were grown. He is also indebted to Dr. H. A. Spoehr, who initiated the work in this laboratory on carbon dioxide absorption by unilluminated leaves, and to Dr. H. H. Strain for many helpful suggestions.

Division of Plant Biology
Carnegie Institution of Washington
Stanford University
Palo Alto, California

LITERATURE CITED

1. Arens, K. Physiologisch polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen. II. Die $Ca(HCO_3)_2$-Assimilation. Jahrb. wiss. Bot. **83**: 513–560. 1936.
2. ———. Manganablagerungen bei Wasserpflanzen als Folge des physiologisch polarisierten Massenaustausches. Protoplasma **30**: 104–129. 1938.
3. Bohr, Christian. Definition und Methode zur Bestimmung der Invasions- und Evasionscoefficienten bei der Auflösung von Gasen in Flüssigkeiten. Werthe der genannten Constanten sowie der Absorptionscoefficienten der Kohlensäure bei Auflösung in Wasser und in Chlornatriumlösungen. Ann. Phys. Chem. **68**: 500–525. 1899.
4. Blackman, F. F. Experimental researches on vegetable assimilation and respiration. II. On the paths of gaseous exchange between

aerial leaves and the atmosphere. Phil. Trans. Royal Soc., London **186B**: 503–562. 1895.

5. BROWN, HORACE T. The principles of diffusion, their analogies and applications. Jour. Chem. Soc. **113**: 559–585. 1918.
6. BROWN, HORACE T., and ESCOMBE, F. Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Phil. Trans. Royal Soc., London **193B**: 223–291. 1900.
7. DUNOYER, L. La Technique du Vide. Les Presses Univ. de France. Paris, 1924, pp. 15–21.
8. FIFE, J. M., and FRAMPTON, V. L. The effect of carbon dioxide upon the pH and certain nitrogen fractions of the sugar-beet plant. Jour. Biol. Chem. **109**: 643–655. 1935.
9. GUGGENHEIM, E. A., and SCHINDLER, T. D. Studies of cells with liquid-liquid junctions. V. Standards for hydrogen ion measurements and the second dissociation constant of phosphoric acid. Jour. Phys. Chem. **38**: 533–541. 1934.
10. ———, and ———. Salt effects on sulfonphthalein indicators. Jour. Phys. Chem. **38**: 543–556. 1934.
11. HARNED, HERBERT S., and EMBREE, NORRIS D. The temperature variation of ionization constants in aqueous solutions. Jour. Amer. Chem. Soc. **56**: 1050–1053. 1934.
12. HUBERT, B. The physical state of chlorophyll in the living plastid. Rec. trav. bot. néerl. **32**: 323–390. 1935.
13. KETELAAR, J. A. A., and HANSON, E. A. Elementary cell and space group of ethyl-chlorophyllide. Nature **140**: 196. 1937.
14. MACINNES, D. A., BELCHER, D., and SHEDLOVSKY, THEODORE. The meaning and standardization of the pH scale. Jour. Amer. Chem. Soc. **60**: 1094–1099. 1938.
15. MARTIN, S. H. The hydrion concentration of plant tissues. IV. The buffer of sunflower hypocotyl. Protoplasma **1**: 522–536. 1927.
16. NIMS, LESLIE FREDERICK. The second dissociation constant of phosphoric acid from 20° to 50°. Jour. Amer. Chem. Soc. **55**: 1946–1951. 1933.
17. PIRSON, A. Ernährungs- und stoffwechselphysiologische Untersuchungen an *Fontinalis* und *Chlorella*. Zeitschr. Bot. **31**: 193–267. 1937.
18. RABINOWITCH, E. Sorption of carbon dioxide by chlorophyll. Nature **141**: 39. 1938.
19. REILLY, J., RAE, W. N., and WHEELER, T. S. Physico-chemical methods, pp. 68–70. D. Van Nostrand Co., Inc., New York. 1927.
20. SEIDELL, ATHERTON. Solubilities of inorganic and organic compounds, Vol. I. p. 211. D. Van Nostrand Co., Inc., New York. 1919.

21. SHEDLOVSKY, THEODORE, and MACINNES, D. A. The first ionization constant of carbonic acid, 0° to 38°, from conductance measurements. Jour. Amer. Chem. Soc. **57**: 1705–1710. 1935.
22. SMITH, JAMES H. C. Carotene IV. The hydrogenation of carotenes obtained from different sources, of dihydrocarotene, and of lycopin. Jour. Biol. Chem. **96**: 35–51. 1932.
23. SPOEHR, H. A. Photosynthesis. Pp. 310–314. The Chemical Catalogue Co., New York. 1926.
24. ————, and MCGEE, J. M. Absorption of carbon dioxide the first step in photosynthesis. Science, n.s., **59**: 513–514. 1924.
25. ————, and ————. The absorption of CO_2 by the leaf material. Year Book, Carnegie Inst. Washington **22**: 53. 1924.
26. ————, and ————. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **23**: 132–133. 1924.
27. ————, and NEWTON, WILLIAM. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **24**: 160–161. 1925.
28. ————, and ————. Absorption of carbon dioxide by leaf material. Year Book, Carnegie Inst. Washington **25**: 177–180. 1926.
29. ————, SMITH, JAMES H. C., STRAIN, H. H., and MILNER, H. W. Absorption of carbon dioxide by the unilluminated leaf. *Cf.* Year Books, Carnegie Institution of Washington **35**: 201–204. 1936; **36**: 203–205, 1937; **37**: 213–215. 1938.
30. THORNTON, NORWOOD C. Carbon dioxide storage. IV. The influence of carbon dioxide on the acidity of plant tissue. Contrib. Boyce Thompson Inst. **5**: 403–418. 1933.
31. VAN SLYKE, DONALD D., and NEILL, JAMES M. The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. Jour. Biol. Chem. **61**: 523–573. 1924. (*Cf.* VAN SLYKE, DONALD D., and SENDROY, JULIUS, JR., Jour. Biol. Chem. **73**: 127–144. 1927.)
32. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR. Untersuchungen über Chlorophyll. Julius Springer, Berlin. 1913. a) Pp. 133–135; b) p. 269.
33. WILLSTÄTTER, RICHARD, and STOLL, ARTHUR. Untersuchungen über die Assimilation der Kohlensäure. Julius Springer, Berlin. 1918. a) pp. 172, 182; b) pp. 179, 192, 198, 217, 223; c) pp. 182, 183, 187; d) pp. 30[illegible], 292; e) p. 416; f) p. 436; g) pp. 298–305.

DISTRIBUTION OF NITROGENOUS AND CARBOHYDRATE FRACTIONS AND OTHER SUBSTANCES IN EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS[1]

C. P. SIDERIS, B. H. KRAUSS, AND H. Y. YOUNG

(WITH NINE FIGURES)

Introduction

After the first fruit on a pineapple is removed, two or more lateral shoots frequently develop from the leaf axis of the mother plant and on these, fruits are borne the following year. It became of interest to study the difference in physiological behavior and chemical composition of such sister shoots, both on the same plant, grown in darkness and seasonal light, respectively, while receiving their entire inorganic nutrition and water supply by way of the roots and stem of the same mother plant. The studies reported below represent an attempt to furnish information on the behavior of pairs of sister shoots so treated. Another objective was to study the transfer of organic and inorganic solutes from the parent stem to the shoots, one grown in darkness and the other under daylight conditions.

BURKHOLDER (5) has satisfactorily reviewed and discussed the literature on the rôle of light in the life of plants. As certain original sources of information bearing directly on this subject are mentioned, however, their data are compared and discussed in connection with the findings reported below. BROWN and TRELEASE (4), working with *Cestrum nocturnum*, observed that the length of shoots of this plant decreased during the day, owing to water loss by transpiration. At night the shoots not only recovered their original length but also made further rapid growth. TRELEASE (31) observed a parallel case in *Musa sapientum*. TOLMACHEV (29) noted in Opuntia sp. that the rate of growth was synchronous with decreased acidity of the tissues. According to this investigator and RICHARDS (19), the acidity of Opuntia sp. was greater during the night than during the day. STREET (27) observed that *Pisum sativum*, grown in water cultures, absorbed more magnesium and calcium when exposed to long than to short periods of light. With respect to nitrate assimilation, NIGHTINGALE and SCHERMERHORN (14), observed in *Asparagus officinalis*, that nitrate was utilized very rapidly both in darkness and in light as long as the tissues were supplied with ample quantities of carbohydrates. TOTTINGHAM and LEASE (30) observed, in the case of *Triticum sativum, Solanum lycopersicum*, and *Lycopersicum esculentum*, that, in blue to violet light, the percentage of protein increased; at the same time there was a decrease in

[1] Published with the approval of the Director as Technical Paper no. 116 of the Pineapple Experiment Station, University of Hawaii.

the amount of pentosans. The conditions favoring the synthesis or hydrolysis of proteins have been studied by different investigators. IVANOFF (10) observed that, for the synthesis of proteins in the absence of light, plants like *Brassica napus, Daucus carota,* and *Solanum tuberosum,* required a small amount of protein and a great supply of amides and readily assimilable carbohydrates. This, in general principle, is in agreement with the findings of most investigators. According to EISENMENGER (7), and KISHI and YOKOTA (12), the absence of light, causing a decrease in the supply of carbohydrates, favors the accumulation of nitrate in the plant and the hydrolysis of proteins; these processes result in an increase in the amino acid nitrogen content of the tissues. With insufficient quantities of light, where a normal rate of carbohydrate synthesis is limited, nitrogen cannot be used effectively by the plant, according to PRIESTLEY (17). Moreover, under conditions of prolonged darkness, proteins are apparently respired as the source of energy, according to NIGHTINGALE, *et al.* (15). PRIESTLEY and EWING (18) attribute the decreased rate of solute movement in the tissues of etiolated plants to structural modifications in the growing point of the plant. In pineapple plants grown in nutrient solutions, greater quantities of proteins and smaller quantities of soluble organic nitrogen were produced when the plants were exposed to light, than when they were kept in darkness, according to certain other studies of the authors (24).

Experimental methods

During the period between the 2nd and 23rd of July, 1934, approximately two weeks after the fruits had been picked, 40 pairs of pineapple sister shoots were selected on the basis of one pair per mother plant. One of the members of each pair was covered with a bag made of light green oil cloth which fitted loosely around the shoot. The lower opening of the bag was partly closed to prevent the entrance of light. The other member of the pair was left exposed to light. These sister shoots will be designated as "covered" and "exposed," respectively.

At intervals of 17, 56, 77 and 105 days from the time that half the shoots were covered, samples consisting of from 5 to 10 pairs of shoots were detached from their mother plants. These shoots were segregated into covered and exposed groups and prepared for analysis. The technique described in a previous publication (22) was employed.

The leaves and stem were separated into homogeneous samples on the basis of age, morphological condition, and physiological function of the various sections of these organs. The chemical methods described in other publications (21, 22) were adopted.

It was of course impossible to detach the growing shoots and obtain their initial weights at the beginning of the experiments. Shoot weights for the

different groups, however, were obtained at the end of each experimental period. The various sections of the leaves and stem of the shoots removed at the end of all the four experimental periods were analyzed for moisture, chlorophyll, electrical resistance, titratable acidity, pH, nitrate nitrogen, reducing sugars and sucrose. The shoots removed at the end of the 56-day period were analyzed also for organic nitrogen fractions. The results from the various studies are presented in tables I to XI and in figures 2 to 9.

Results

Weights

The weights of the shoots from different plants varied somewhat, as far as we could determine by observation, at the time of selection although particular attention was paid to the uniformity of the pair of shoots on each mother plant. Because of inherent variability some pairs of shoots were smaller than other pairs, and the degree of variability from the mean weight was greater than 20 per cent. for the shoots removed at the end of the different experimental periods. Average shoot weights are presented in table I.

TABLE I

WEIGHT OF EXPOSED AND COVERED PINEAPPLE SHOOTS REMOVED FROM THEIR MOTHER PLANTS AT THE END OF VARIOUS PERIODS OF TIME

Period	Weight of shoots	
	Exposed	Covered
days	*gm.*	*gm.*
17	618	635
56	980	1010
77	1303	1110
105	1465	921

The data suggest that pineapple shoots kept in darkness by covering for a period of one to two months, may grow without any external symptoms of detriment when attached to the mother plant. Under the longer experimental periods, however, when the shoots were allowed to remain on the plants for 77 and 105 days, the lesser weights of the covered shoots represent a decreased rate of growth caused by the effects of shading. The losses in weight of the shoots during these two periods were accompanied also with more pronounced morphological and physico-chemical changes in the leaves and stem, as presented later.

Moisture

The moisture content of the various sections of the leaves and of the stem of the covered and exposed shoots removed at the end of all the four experi-

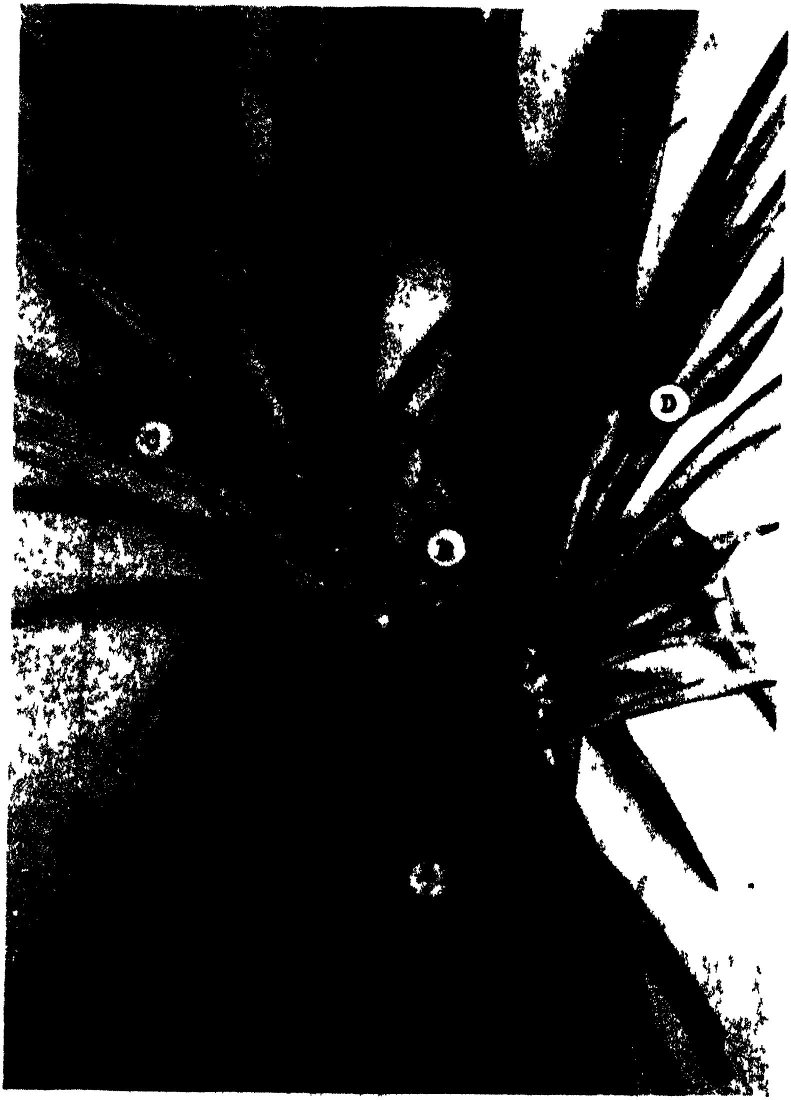


FIG. 1. Mother plant (A) with the peduncle (B) of the first season's fruit, and two shoots (C and D) which will produce the second season's fruit.

mental periods are presented in table II. Figure 2 illustrates the distribution of moisture in the sections of the exposed and covered shoots removed at the end of the 105-day period.

The data show, in general, that the moisture percentage of the tissues of the exposed shoots decreased with advancing age while that of comparable

TABLE II

MOISTURE CONTENT OF VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

PLANT SECTIONS	PERIODS							
	17 DAYS		56 DAYS		77 DAYS		105 DAYS	
	EXPOSED	COVERED	EXPOSED	COVERED	EXPOSED	COVERED	EXPOSED	COVERED
Leaves: Old:	%	%	%	%	%	%	%	%
B1 (base)	86.4	87.3	87.0	86.8	85.7	87.6	87.5	90.1
B2	85.3	85.3	83.2	83.0	84.9	85.6	86.0	88.2
B3	84.7	86.7	83.4	84.7	84.1	85.4	85.0	87.1
B4 (tip)			82.1	81.3	82.3	76.5	81.5	29.6*
Mature:								
C1 (base)	92.4	92.5	89.7	90.2	89.9	89.8	90.7	91.8
C2	88.4	90.3	85.8	87.0	86.5	87.2	88.1	89.8
C3	85.0	89.2	83.1	89.3	84.7	89.0	85.4	90.4
C4			82.0	89.0	84.2	88.3	82.7	90.0
C5 (tip)			82.3	88.2	81.1	85.4	78.4	81.2
Active:								
D1 (base)			91.4	90.8	91.2	92.4	92.0	93.0
D2			88.0	89.8	88.2	89.3	88.4	90.6
D3			84.8	89.2	86.8	90.4	85.2	91.4
D4			83.7	90.1	84.2	90.3	84.6	91.2
D5 (tip)			83.5	88.2	81.5	88.0	79.6	89.6
Young:								
E1 (base)			91.4	93.6	92.1	92.2	92.0	92.6
E2			87.8	88.8	88.1	90.0	88.3	89.6
E3			87.0	88.4	88.4	90.4	86.0	90.2
E4 + 5 (tip)			84.5	88.4	84.1	89.6	83.5	89.6
Stem:								
Basal (cortex)	75.5	79.6		86.4	85.8	86.6	89.0	89.2
" (pith)	76.1	77.8	78.9	-	82.2	85.0	85.2	90.3
Apical (cortex)	87.3	79.1	83.7	87.6	87.5	89.5	90.0	90.2
" (pith)	86.3	87.6	84.5	88.1	85.8	89.2	87.2	90.6

* Tissues dead and possibly dried.

tissues of the covered shoots increased. This condition indicates that the amounts of solids per unit of fresh weight were greater in the tissues of the exposed than in those of the covered shoots.

CHLOROPHYLL

The chlorophyll content of the sections of leaves of both exposed and covered shoots removed at the end of all the four periods is presented in table III. Figure 3 reports only the chlorophyll content of the leaf sections of covered and exposed shoots removed at the end of the 56-day period.

The data in table III show that the amounts of chlorophyll in the leaves

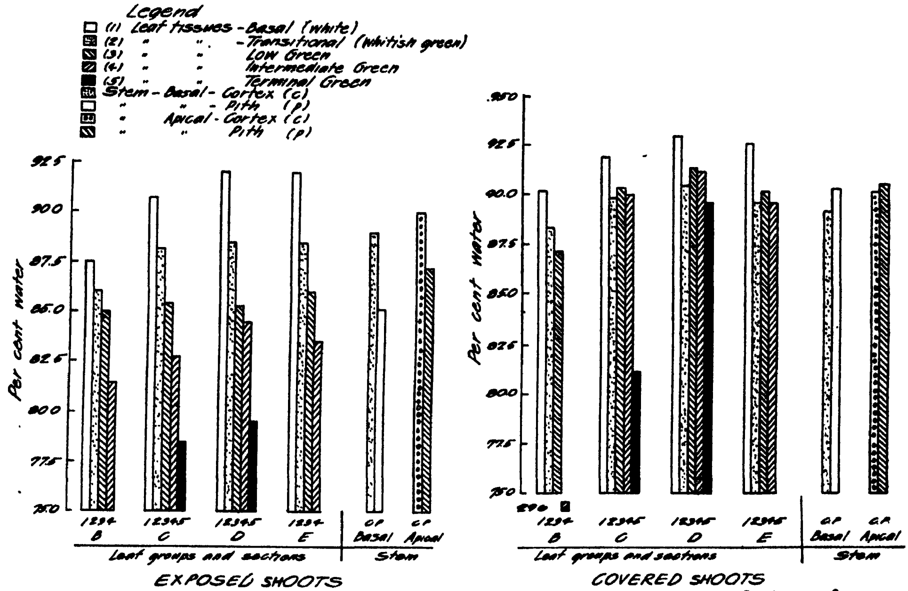


FIG. 2. Percentage of water in different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 105-day period.

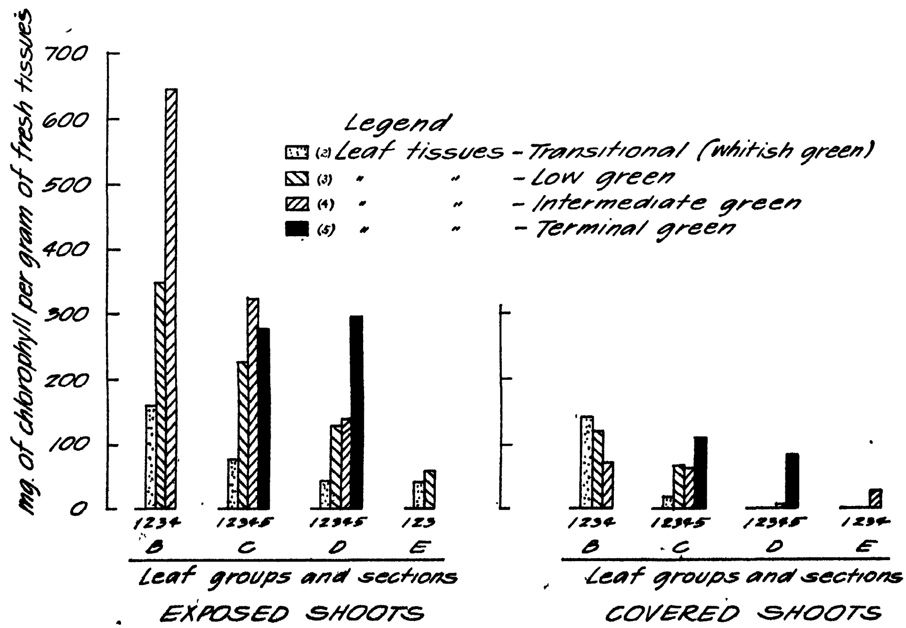


FIG. 3. Milligrams of chlorophyll per gram of fresh weight in different sections of the leaves of exposed and covered pineapple shoots at the end of the 56-day period.

TABLE III

MILLIGRAMS OF CHLOROPHYLL PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THE MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

PLANT SECTIONS	PERIODS							
	17 DAYS		56 DAYS		77 DAYS		105 DAYS	
	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED
Leaves:	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Old:								
B1 (base)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B2	0.122	0.150	0.162	0.148		0.040	0.124	
B3	0.553	0.518	0.346	0.126	0.388	0.149	0.299	0.038
B4 (tip)			0.718	0.072	0.390	0.306	0.592	0.000
Mature:								
C1 (base)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C2	0.131	0.050	0.071	0.017		0.000	0.100	0.000
C3	0.297	0.232	0.220	0.078	0.258	0.000	0.346	0.000
C4			0.320	0.075	0.396	0.093	0.441	
C5 (tip)			0.272	0.102	0.290	0.257	0.557	
Active:								
D1 (base)			0.000	0.000	0.000	0.000	0.000	0.000
D2			0.035	0.000	0.073	0.000	0.075	0.000
D3			0.126	0.000	0.203	0.000	0.206	0.000
D4			0.128	0.007		0.000	0.340	0.000
D5 (tip)			0.294	0.082	0.287	0.160	0.478	
Young:								
E1 (base)			0.000	0.000	0.000		0.000	0.000
E2			0.038	0.000			0.041	0.000
E3			0.048	0.000	0.075		0.162	0.000
E4 + 5 (tip)				0.020	0.400		0.301	0.000

of the covered plants decreased with prolonged darkness; *i.e.*, during a period of 105 days. The low chlorophyll values for the covered shoots during the various periods resulted chiefly from the inability of the plant to produce this substance in the new leaves formed after covering, and also to some losses caused by the breaking down of chlorophyll in the older leaves. The terminal sections of the leaves of the covered shoots removed at the end of the 56-day period contained less amounts of chlorophyll than the same sections of leaves from covered shoots removed at the end of the 77-day period. The greater amounts in the shoots removed at the end of the latter period may indicate variations in the initial chlorophyll content of the experimental material, a state which occurs even under similar nutritional and environmental conditions. It is safe to assume, therefore, that after a very prolonged period of darkness, such as 105 days, the chlorophyll of pineapple leaves undergoes from partial to complete decomposition.

Electrical resistance

The electrical resistance of the extracted sap was determined with a set-up of standard Leeds and Northrup instruments consisting of a student potentiometer, a 6-dial resistance box, an a. c. pointer galvanometer and a Henry-type conductivity cell. The results obtained for the shoots removed at the end of the four different periods are presented in table IV. Figure 4 reports only the data for the shoots removed at the end of the 56-day period.

The data in the table show that the electrical resistance of the sap of the tissues in the covered shoots increased with longer periods of darkness. The electrical resistance values of the terminal sections of the leaves were influenced more by the effects of darkness than the basal sections. The sections

TABLE IV

Electrical resistance of the extracted sap of various leaf and stem sections of exposed and covered pineapple sister shoots attached to their mother plant for different periods of time

Plant sections	Periods: 17 days		56 days		77 days		105 days	
	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
Leaves:	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*	*ohms*
Old:								
B1 (base)	344	350	167	182	116	......	116	112
B2	332	324	157	182	109	......	95	145
B3	220	280	117	200	73	......	87	203
B4 (tip)	......	......	73	157	72	......	65	......
Mature:								
C1 (base)	262	320	100	147	87	......	109	87
C2	294	312	117	178	99	......	95	138
C3	240	292	105	178	69	......	73	124
C4	......	......	88	160	60	......	61	137
C5 (tip)	......	......	75	146	47	......	52	119
Active:								
D1 (base)	......	......	88	186	50	......	93	105
D2	......	......	109	182	85	......	102	115
D3	......	......	134	178	87	......	77	145
D4	......	......	134	178	61	......	58	122
D5 (tip)	......	......	91	109	52	......	51	113
Young:								
E1 (base)	......	......	97	112	67	......	95	87
E2	......	......	145	156	......	......	122	160
E3	......	......	128	165	102	......	128	131
E4 + 5 (tip)	......	......	......	195	87	......	68	135
Stem:								
Basal (cortex)	260	240	102	93	57	......	63	73
" (pith)	218	206	88	78	46	......	50	48
Apical (cortex)	......	......	80	78	58	......	60	53
" (pith)	......	......	60	70	47	......	48	58

of the stem of both covered and exposed shoots do not show as great contrasts as do those of the leaves.

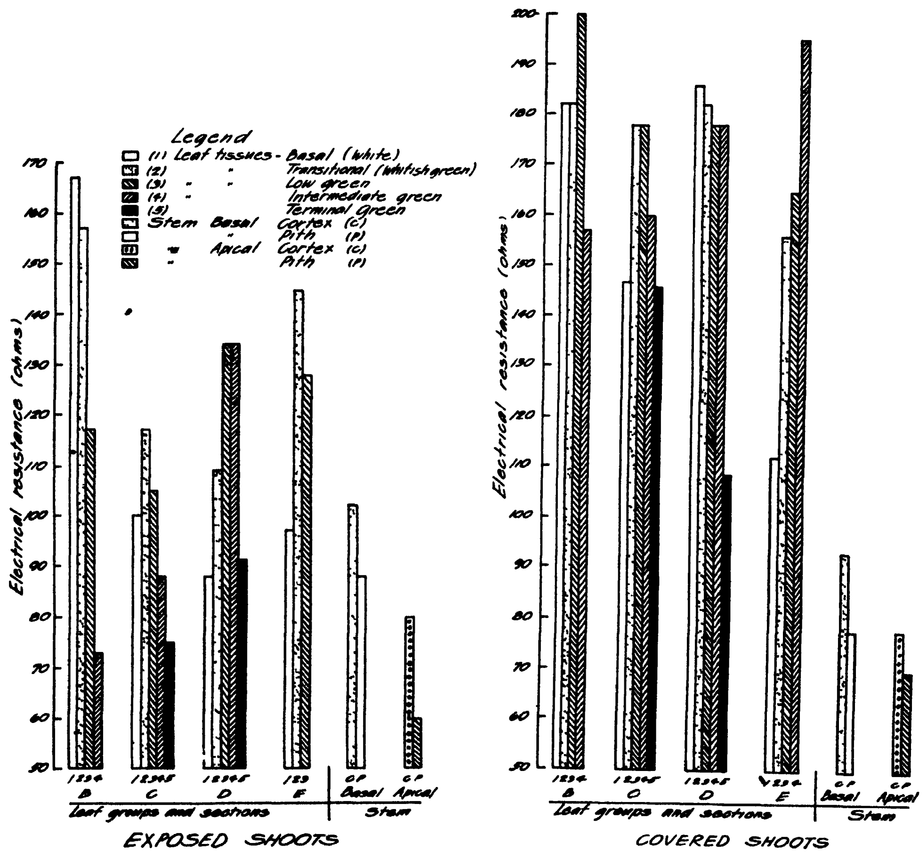


FIG. 4. Electrical resistance (ohms) of the extracted sap of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56-day period.

ACIDITY

Determinations of titratable acidity were made on aliquots from aqueous solutions of the extracted sap from 10-gram samples of fresh tissues. A different aliquot from the same aqueous extract was used for the colorimetric determination of the pH values. The data for titratable acidity for the covered and exposed shoots, removed at the end of all four periods, are presented in table V and in figure 5. The values for pH are reported in table VI and figure 6.

Titratable acidity, reported as percentage of citric acid, increased in the chlorophyllous sections of the exposed shoots with advancing age, while it

TABLE V

TITRATABLE ACIDITY AS CITRIC ACID ON A FRESH WEIGHT BASIS IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

PLANT SECTIONS	PERIODS							
	17 DAYS		56 DAYS		77 DAYS		105 DAYS	
	EXPOSED	COVERED	EXPOSED	COVERED	EXPOSED	COVERED	EXPOSED	COVERED
Leaves:	%	%	%	%	%	%	%	%
Old:								
B1 (base)	0.192	0.192	0.038	0.093	0.064	....	0.045	0.090
B2	0.179	0.179	0.051	0.064	0.160	....	0.154	0.025
B3	1.536	0.486	0.435	0.154	0.864	....	0.813	0.064
B4 (tip)	....	....	0.832	0.333	0.960	....	1.197	....
Mature:								
C1 (base)	0.224	0.179	0.179	0.218	0.160	....	0.090	0.173
C2	0.243	0.154	0.102	0.090	0.060	....	0.190	0.090
C3	1.677	0.333	0.525	0.064	0.960	....	1.114	0.090
C4	....	....	1.254	0.640	1.824	....	1.818	0.173
C5 (tip)	....	....	1.472	0.256	2.110	....	1.920	0.282
Active:								
D1 (base)	....	....	0.179	0.100	0.320	....	0.128	0.154
D2	....	....	0.128	0.128	0.160	....	0.109	0.064
D3	....	....	0.540	0.512	0.448	....	0.813	0.090
D4	....	....	1.090	0.461	1.660	....	1.837	0.154
D5 (tip)	....	....	1.357	0.320	2.050	....	2.138	0.282
Young:								
E1 (base)	....	....	0.154	0.448	0.224	....	0.090	0.128
E2	....	....	0.102	0.077	....	....	0.109	0.045
E3	....	....	0.435	0.090	0.160	....	0.538	0.064
E4 + 5 (tip)	....	....	....	0.102	0.960	....	1.709	0.128
Stem:								
Basal (cortex)	0.333	0.352	0.179	0.256	0.224	....	0.154	0.256
" (pith)	0.435	0.422	0.192	0.358	0.320	....	0.192	0.301
Apical (cortex)	....	....	0.256	0.550	0.320	....	0.256	0.429
" (pith)	....	....	0.282	0.717	0.480	....	0.256	0.384

decreased in comparable leaf sections of the covered shoots, as shown in table V. The stem and the non-chlorophyllous basal section of the leaves of the covered shoots contained greater amounts of titratable acidity than those of the exposed shoots.

The values for pH presented in table VI and in figure 6 follow more or less the course of titratable acidity. They are high in the basal non-chlorophyllous and low in the chlorophyllous sections of the leaves of the exposed shoots, while in the covered shoots the pH values of the different leaf sections approach a constant level as the total time of shading increased. If we contrast the curves for the pH values of comparable sections of the leaves of exposed and covered shoots we find that in the former the values decrease from the basal to the apical sections, while in the latter they fluctuate within narrow limits.

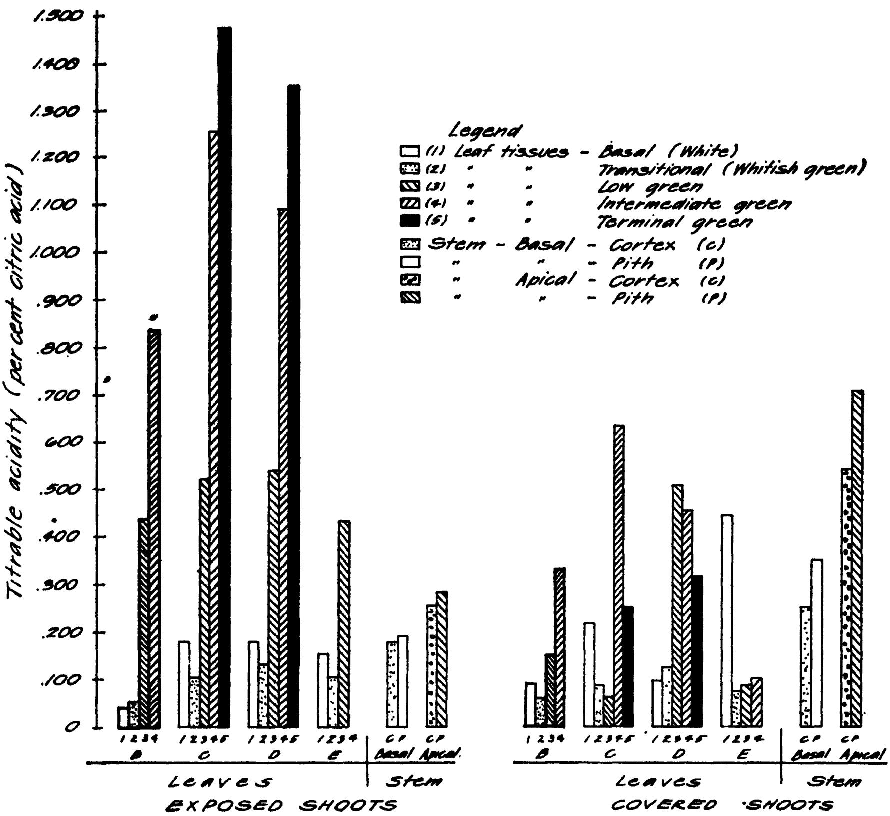


FIG. 5. Titratable acidity as percentage of citric acid on a fresh weight basis of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56-day period.

DISTRIBUTION OF NITROGENOUS FRACTIONS

The distribution of nitrate in the various sections of the leaves of the exposed and covered lots of shoots, removed at the end of all the four periods, are reported in table VII. Table VIII contains the analytical data for the fractions of inorganic, soluble organic, and protein nitrogen in the different sections of the leaves and stem of the exposed and covered shoots removed at the end of the 56-day period. The data in table VIII are presented in graphic form in figures 7 and 8.

The values in table VII show, in general, that the movement of nitrate from the mother plant to the attached sister shoots was greater for the exposed than for the covered shoots. In practically all cases, the amounts of nitrate in the non-chlorophyllous sections of the mature C, active D, and young E groups of leaves were greater for the exposed than for the covered

TABLE VI

HYDROGEN-ION CONCENTRATION OF THE EXTRACTED SAP OF VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

PLANT SECTIONS	PERIODS							
	17 DAYS		56 DAYS		77 DAYS		105 DAYS	
	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED
Leaves:								
Old:	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
B1 (base)	5.4	5.5	4.9	3.8	5.6	......	5.0	5.0
B2	4.7	5.5	5.2	4.8	5.0	......	4.8	5.0
B3	4.0	5.1	4.3	3.8	4.2	......	4.0	4.8
B4 (tip)	...	......	4.2	4.6	4.2	......	4.2	......
Mature:								
C1 (base)	5.6	5.7	5.4	5.0	5.0	......	5.0	5.0
C2	4.8	5.7	5.0	5.1	5.0	......	4.6	5.2
C3	3.9	5.0	4.0	5.5	4.2	......	4.2	5.2
C4	......	......	3.6	3.8	3.8	......	4.0	5.2
C5 (tip)	......	......	3.7	5.2	4.0	......	4.0	5.2
Active:								
D1 (base)	......	......	5.5	5.4	5.2	......	5.2	5.3
D2	......	......	5.4	4.8	5.2	......	5.0	5.6
D3	......	......	4.4	3.6	4.4	......	4.2	5.2
D4	......	......	3.8	4.3	3.7	......	3.8	5.2
D5 (tip)	......	......	3.6	5.0	3.7	......	3.9	5.2
Young:								
E1 (base)	......	......	5.6	4.1	5.4	......	5.5	5.5
E2	......	......	5.4	5.6	......	......	4.8	5.4
E3	......	......	4.2	5.4	4.7	......	4.2	5.3
E4 + 5 (tip)	......	......	......	5.4	4.0	......	3.8	5.2
Stem:								
Basal (cortex)	5.6	5.3	4.8	4.8	5.0	......	5.0	5.0
" (pith)	5.5	5.5	5.1	5.0	5.0	......	5.2	5.0
Apical (cortex)	......	......	5.2	5.0	5.2	......	5.2	5.0
" (pith)	......	......	5.4	4.8	5.2	......	5.2	5.2

shoots. Exceptions may be found in the basal sections of the senile B group of leaves, and in the stem. The rate of nitrate assimilation, as indicated by the difference between the amounts of this substance in the basal non-chlorophyllous sections (no. 1) and in the chlorophyllous sections (no. 3 or 4) of the C, D, and E groups of leaves, is considerably greater for the exposed than for the covered shoots. The causes for the low rate of nitrate assimilation in the leaves of the covered shoots may be attributed to the small amounts of chlorophyll and carbohydrate in the leaves and possibly unknown substances.

Certain differences that may be observed in the amounts of nitrate in the non-chlorophyllous sections of both covered and exposed lots of sister shoots at the end of the different experiment periods were caused by the length of

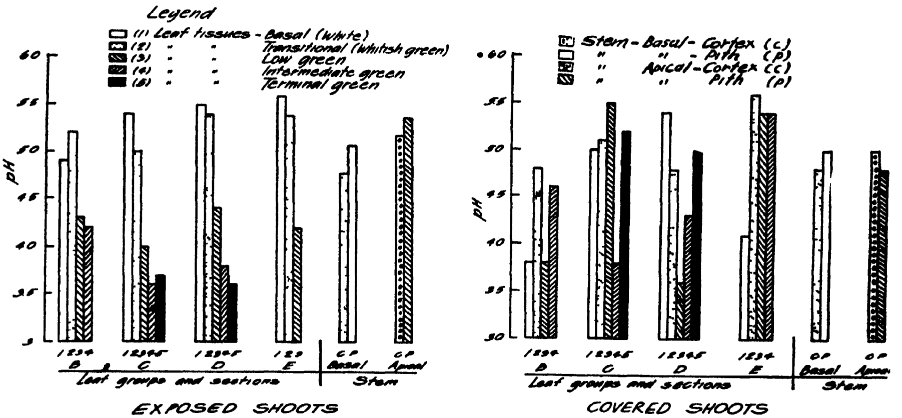


FIG. 6. pH of the extracted sap of different sections of the leaves and stem of exposed and covered pineapple shoots at the end of the 56 day period.

TABLE VII

MILLIGRAMS OF NITRATE NITROGEN PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME

PLANT SECTIONS	PERIODS							
	17 DAYS		56 DAYS		77 DAYS		105 DAYS	
	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED	EX-POSED	COV-ERED
Leaves:	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Old:								
B1 (base)	0.310	0.185	0.357	0.292	0.194	0.169	0.495	0.520
B2	0.231	0.174	0.237	0.184	0.060	0.068	0.316	0.433
B3	0.007	0 000	0.031	0.036	0.012	0.044	0.019	0.163
B4 (tip)			0.008	0.000	0.007	0.019	0.004	
Mature:								
C1 (base)	0.338	0.105	0.394	0.109	0.427	0.066	0.672	0.163
C2	0.091	0.105	0.356	0.112	0.257	0.072	0.427	0.178
C3	0.003	0.025	0.025	0.106	0.014	0.058	0.012	0.150
C4			0.008	0.042	0.000	0.057	0.002	0.116
C5 (tip)			0.000	0.006	0.000	0.022	0.000	0.079
Active:								
D1 (base)			0.465	0.069	0.267	0.058	0.642	0.061
D2			0.310	0.056	0.267	0.023	0.427	0.037
D3			0.089	0.032	0.044	0.031	0.020	0.024
D4			0.021	0.029	0.000	0.017	0.002	0.021
D5 (tip)			0.000	0.026	0.000	0.023	0.000	0.026
Young:								
E1 (base)			0.205	0.080	0.151	0.039	0.357	0.046
E2			0.033	0.042	0.116	0.028	0.171	0.033
E3			0.019	0.026	0.060	0.025	0.015	0.021
E4 + 5 (tip)			0.000	0.025	0.000	0.028	0.005	0.022
Stem:								
Basal (cortex)	0.149	0.185	0.347	0.530	0.538	0.237	0.608	0.588
" (pith)	0.268	0.143	0.131	0.440	0.567	0.243	0.924	0.588
Apical (cortex)	0.350	0.366	0.356	0.257	0.231	0.170	0.463	0.562
" (pith)	0.535	0.544	0.144	0.300	0.280	0.200	0.602	0.575

TABLE VIII

MILLIGRAMS PER GRAM OF FRESH WEIGHT OF INORGANIC, SOLUBLE ORGANIC, AND PROTEIN NITROGEN FRACTIONS IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR 56 DAYS

PLANT SECTIONS	MILLIGRAMS OF NITROGEN PER GRAM OF FRESH TISSUE													
	EXPOSED SHOOTS							COVERED SHOOTS						
	INORGANIC		SOLUBLE ORGANIC				PROTEIN	INORGANIC		SOLUBLE ORGANIC				PROTEIN
	AMMONIUM*	NITRATE	AMIDE	ALPHA-AMINO	REST SOLUBLE	TOTAL		AMMONIUM	NITRATE	AMIDE	ALPHA-AMINO	REST SOLUBLE	TOTAL	
Leaves:	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Old:														
B1 (base)	0.0056	0.357	..	0.274	0.370	...	0.325	0.003	0.292	0.040	0.270	0.200	0.510	0.168
B2	0.0029	0.237	0.014	0.295	0.195	0.504	0.420	0.014	0.184	0.014	0.227	0.165	0.406	0.112
B3	0.0067	0.031	0.017	0.405	0.559	0.981	0.840	0.027	0.036	0.030	0.392	0.295	0.717	0.428
B4 (tip)	...	0.008	0.235	0.890	1.180	2.305	1.240	0.065	0.000	0.060	0.567	0.633	1.260	0.614
Mature:														
C1 (base)	0.0052	0.394	0.070	0.340	0.660	1.070	0.368	0.065	0.109	0.067	0.480	0.360	0.907	0.245
C2	0.0070	0.356	0.056	0.406	0.266	0.728	0.502	0.046	0.112	0.032	0.338	0.124	0.494	0.350
C3	0.0029	0.025	0.056	0.548	0.438	1.042	0.646	0.050	0.106	0.033	0.468	0.280	0.781	0.394
C4	...	0.008	0.112	0.736	0.748	1.596	0.870	0.035	0.042	0.034	0.459	0.381	0.874	0.516
C5 (tip)	...	0.000	0.168	1.020	1.480	2.668	0.870	0.075	0.006	0.018	0.540	0.860	1.418	0.525
Active:														
D1 (base)	...	0.465	...	0.684	0.519	...	0.313	0.022	0.069	0.040	0.503	0.487	1.030	0.327
D2	...	0.310	.	0.548	0.292	...	0.340	0.011	0.056	0.018	0.432	0.128	0.578	0.310
D3	...	0.089	...	0.428	0.272	...	0.438	0.012	0.032	0.046	0.393	0.167	0.606	0.348
D4	...	0.021	..	0.428	0.412	...	0.682	0.041	0.029	0.092	0.439	0.275	0.806	0.464
D5 (tip)	...	0.000	.	0.570	0.810	..	0.885	0.023	0.026	0.134	0.540	0.650	1.324	...
Young:														
E1 (base)	...	0.205	..	1.080	0.404		0.400	0.003	0.080	0.119	0.810	0.670	1.599	...
E2	...	0.033	..	0.970	0.332		0.317	0.019	0.042	0.046	0.548	0.224	0.818	...
E3	...	0.019		0.658	0.274		0.514	0.012	0.026	0.046	0.720	.	...	0.347
E4 + 5 (tip)	...	0.000		...	..		.	0.012	0.026	0.046	0.470	0.090	0.606	0.206
Stem:														
Basal (cortex)	...	0.347		1.190	0.070		1.225	0.011	0.530	0.080	0.712	0.110	0.902	...
" (pith)	...	0.131	.	0.822	1.978		1.960	0.014	0.440	0.050	0.674	0.901	1.625	0.467
Apical (cortex)	...	0.356	0.028	0.862	0.351	1.241	0.850	0.023	0.257	0.059	0.988	1.212	2.259	0.805
" (pith)	...	0.144	...	0.988	0.992		1.060	0.050	0.300	0.077	1.280	0.905	2.262	0.710

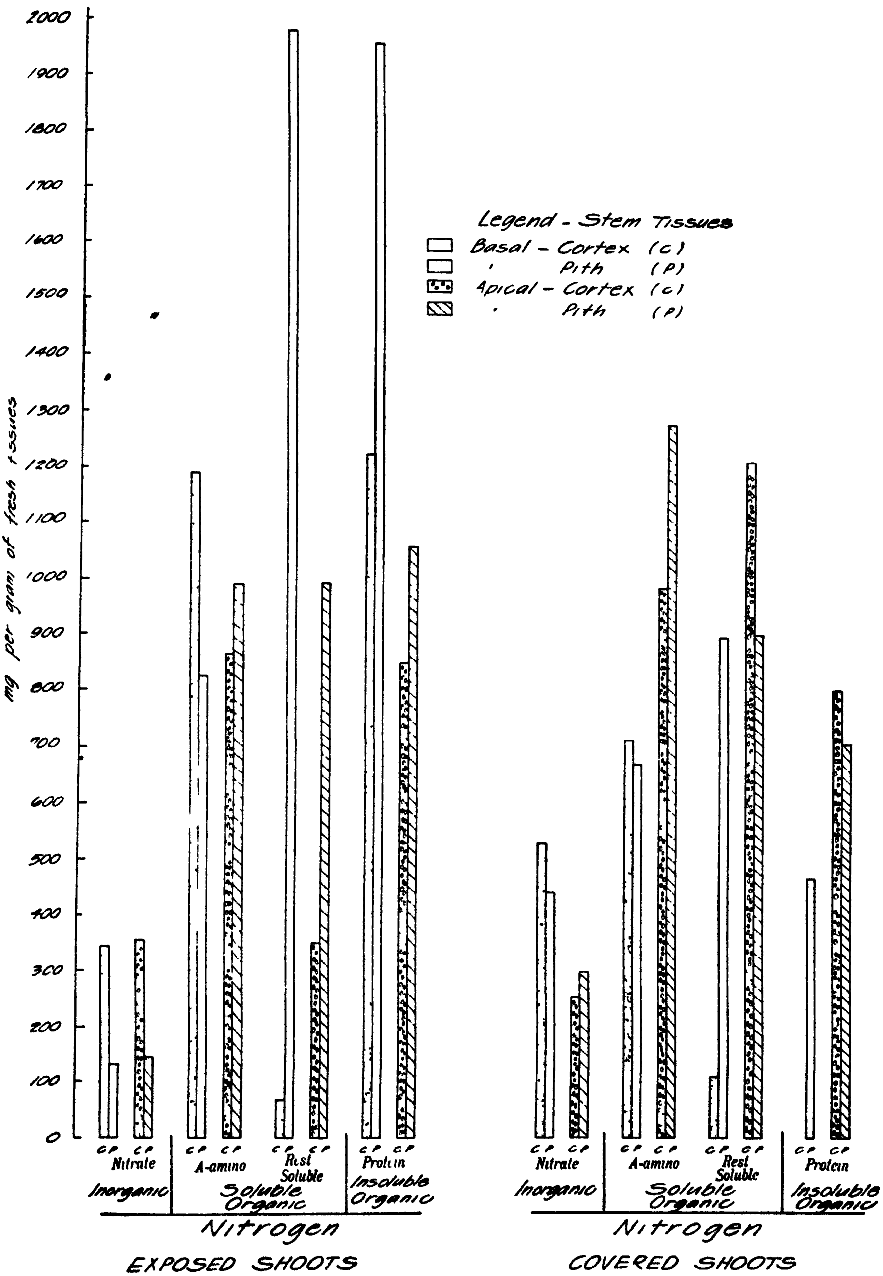


FIG. 7. Milligrams of different fractions of inorganic, soluble organic, and protein nitrogen per gram of fresh weight in different sections of the stem of exposed and covered pineapple shoots at the end of the 56-day period.

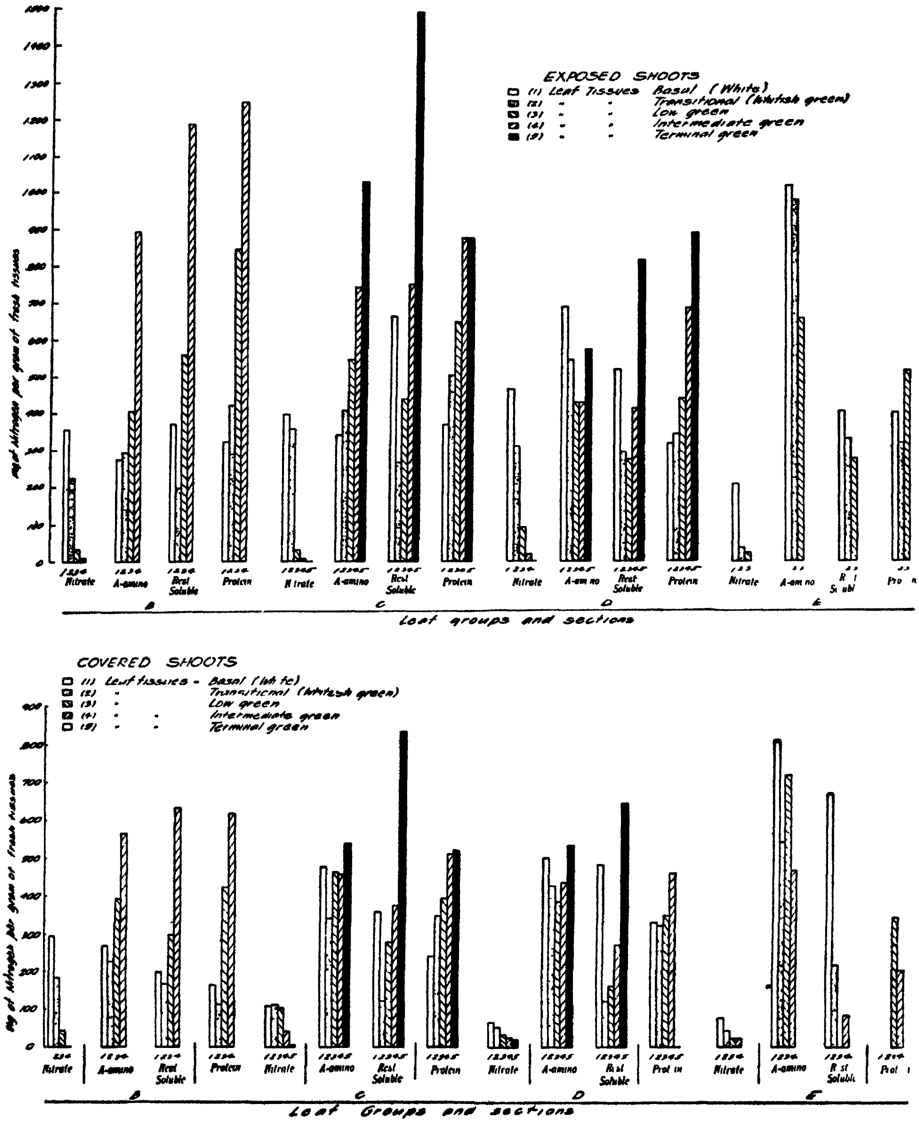


FIG. 8. Milligrams of different fractions of inorganic, soluble organic, and protein nitrogen per gram of fresh weight in different sections of the leaves of groups B, C, D, and E of exposed (A) and covered (B) pineapple shoots at the end of the 56-day period.

the interval between applications of fertilizer salts and time of harvesting. The results show, in general, that in pineapple leaves devoid of chlorophyll, nitrate is not reduced and converted to organic nitrogen compounds at the same rate that it is reduced and converted in chlorophyllous leaves. With

respect to the distribution of inorganic and organic nitrogen in the exposed and covered shoots removed at the end of the 56-day period, the data in table VIII show the following conditions:

1. Ammonium was present in relatively small amounts in the various sections of the leaves and stem of the covered and exposed shoots. The distribution of nitrate in the two lots of shoots and the conditions favoring or inhibiting its assimilation have been discussed.

2. The amounts of soluble amide nitrogen (glutamine and asparagine) increased from the transitional subchlorophyllous (no. 2) section to the terminal sections of the leaves of groups B and C of the exposed shoots. In the covered shoots this trend was not well pronounced. The basal nonchlorophyllous sections contained relatively larger amounts of amide nitrogen than the transitional sections immediately above. The quantities of amide nitrogen in the D group of leaves of the covered shoots increased from the transitional (no. 2) to the terminal (no. 5) section. In the E group of leaves of the covered shoots the amounts of amide nitrogen were relatively great in the basal section, while in the transitional and chlorophyllous sections they were appreciably smaller.

There are no data for the amide nitrogen content of the leaf groups D and E of the exposed plants because of the accidental loss of samples.

3. Alpha-amino nitrogen was relatively very abundant in most sections of the leaves and of the stem. Its distribution in the mature leaves of exposed shoots increased from the basal (no. 1) to the terminal (nos. 4 and 5) sections. In comparable sections of the leaves of the covered shoots, the trend of this distribution was irregular. In the active D and in the young E groups of leaves of the exposed shoots, where production of new tissues had not reached completion, more alpha-amino nitrogen accumulated in the basal than in the terminal sections. In the D and E groups of leaves of the covered shoots the trend of alpha-amino nitrogen distribution was practically the same as in the exposed shoots, excepting that the quantities were relatively smaller. The amounts of alpha-amino nitrogen in the cortex of the mature and aged basal section of the stem of the exposed shoots were greater than those in the cortex of the young and growing apical section, indicating that alpha-amino nitrogen was utilized more rapidly in the growing than in the mature tissues of the stem. In the covered shoots the amounts of alpha-amino nitrogen in the cortex of the stem were generally greater in the apical than in the basal sections, indicating accumulations in the former tissues resulting possibly from a retardation in the normal processes of new growth due to covering. The amount of alpha-amino nitrogen in the pith of the stems of both the exposed and covered shoots was greater in the apical than in the basal section, being practically twice as great in the apical section of the covered shoots as in the basal section. These results

indicate possibly either an interruption in the movement of this substance to other sections of the plant or non-utilization resulting from retarded growth activity.

4. Rest soluble organic nitrogen, consisting of various unidentified nitrogenous compounds, was present in comparatively great quantities in both exposed and covered shoots. In the B and C groups of leaves of both lots of shoots, rest soluble organic nitrogen increased from the transitional subchlorophyllous (no. 2) to the terminal (nos. 4 and 5) sections. In the basal non-chlorophyllous (no. 1) sections the quantities of rest soluble organic nitrogen were greater than in the transitional (no. 2) sections immediately above. The amounts of this nitrogen fraction were consistently greater in the sections of the leaves of the exposed than in those of the covered shoots.

In the exposed lots of shoots the amounts of rest soluble organic nitrogen were less in the terminal chlorophyllous sections of the more active D group of leaves, possibly because they were utilized more rapidly for new growth in the meristematic tissues of the basal (no. 1) sections, than in comparable sections of such groups of leaves as B and C, which had apparently completed their growth. In the terminal sections of the young E group of leaves of both lots of shoots, the amounts of rest nitrogen were very low, indicating a much higher rate of utilization, and consequently of growth, in the meristematic tissues of the basal sections of this group (E) than in those of the D group of leaves. It will be observed that the value for rest soluble organic nitrogen in the basal non-chlorophyllous (no. 1) section of the young E group of leaves of the covered shoots, composed mostly of meristematic tissues, was considerably higher than for the corresponding section of the exposed shoots, indicating probably that the rate of utilization was not as great in the former as it was in the latter.

The distribution of rest soluble organic nitrogen varied in the cortex and pith of the two sections of the stem of the exposed and covered shoots. The amounts were small in the cortex of the basal section and great in the upper or apical section of the stem of both lots of shoots. The pith of both the apical and basal stem sections of both lots of shoots contained relatively great quantities of rest soluble organic nitrogen.

5. Protein nitrogen accumulated in the various sections of practically all groups of leaves of the exposed shoots in amounts approximately 1.5 to 2.0 times greater than those found in comparable sections of the leaves of the covered shoots. The comparatively greater amounts of chlorophyll in the exposed than in the covered shoots and also the more favorable conditions for photosynthesis and carbohydrate accumulation for the former than for the latter shoots were doubtless factors which also favored the synthesis of proteins. Protein accumulated in greater amounts in the pith of both the apical and basal sections of the stem of the exposed than of the covered

shoots. The difference between the amounts of protein in the cortex of the apical sections of the exposed and covered shoots was very small. In the absence of similar data for the cortex of the basal section of the stem of the covered shoots no contrasts can be made between this portion of the two lots of shoots. If we contrast the distribution of protein in the different sections of the stem of the exposed shoots we find that it is greater in the cortex of the basal than in that of the apical section of the stem, indicating possible storage of this substance in tissues which had completed growth.

Distribution of sugars

Reducing sugars were determined by Bertrand's method as presented by Kertesz (11). Sucrose was determined after hydrolysis with invertase.

TABLE IX

Amount of reducing sugars in different sections of the leaves and stem of exposed and covered pineapple sister shoots attached to their mother plant for different periods of time. Based on percentage of fresh weight

Plant sections	Periods							
	17 days		56 days		77 days		105 days	
	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
Leaves:	%	%	%	%	%	%	%	%
Old:								
B1 (base)	2.12	2.09	2.36	2.44	2.58	2.07	1.90	1.81
B2	2.14	2.00	2.42	2.35	2.93	2.11	1.85	1.68
B3	1.59	1.16	1.62	2.18	2.03	2.10	1.76	1.62
B4 (tip)			1.52	2.12	1.90	1.71	1.47	
Mature:								
C1 (base)	1.50	2.00	2.11	2.44	2.61	2.23	2.03	1.77
C2	1.70	1.83	1.94	2.22	2.55	2.15	1.89	1.98
C3	1.80	1.62	1.69	1.87	2.04	1.90	1.41	1.62
C4			1.85	2.01	1.92	1.98	1.45	1.50
C5 (tip)			2.00	2.05	1.87	1.92	1.85	2.31
Active:								
D1 (base)			1.44	2.01	1.59	2.11	1.91	1.60
D2			1.44	1.99	1.89	1.89	1.60	1.82
D3			1.13	1.73	1.96	1.73	1.81	1.49
D4			1.64	1.75	2.13	1.77	1.83	1.53
D5 (tip)			2.12	1.85	2.60	2.00	1.63	1.52
Young:								
E1 (base)			1.73	1.44	1.64	1.28	1.80	1.08
E2			1.78	1.95	1.73	1.90	1.65	2.01
E3			2.01	1.84	1.80	1.70	2.11	1.87
E4 + 5 (tip)				1.95	2.52	1.54	2.17	2.01
Stem:								
Basal (cortex)	1.95	1.86	2.11	1.84	1.90	1.64	1.34	1.17
" (pith)	1.41	2.11	1.75	1.90	0.93	0.64	0.68	0.76
Apical (cortex)		1.95	1.84	1.63	2.01	1.40	0.83	0.73
" (pith)		1.34	0.92	0.95	0.92	0.67	0.25	0.27

The distribution of reducing sugars in the various sections of the leaves and stem of exposed and covered shoots removed at the end of all the four different experimental periods is reported in table IX, and that of sucrose in table X. The data on reducing and total sugars and sucrose for the exposed and covered shoots removed at the end of the 56-day period are presented graphically in figure 9.

TABLE X

AMOUNT OF SUCROSE IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SISTER SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR DIFFERENT PERIODS OF TIME. GIVEN IN PERCENTAGE OF FRESH WEIGHT

Plant sections	Periods							
	17 days		56 days		77 days		105 days	
	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
Leaves:	%	%	%	%	%	%	%	%
Old:								
B1 (base)	0.43	0.35	0.33	0.29	0.35	0.26	0.24	0.25
B2	0.35	0.35	0.34	0.38	0.55	0.33	0.35	0.22
B3	0.83	0.51	0.59	0.52	0.63	0.64	0.74	0.33
B4 (tip)	...	...	0.77	0.65	0.93	0.70	1.08	...
Mature:								
C1 (base)	0.35	0.35	0.27	0.30	0.40	0.29	0.32	0.28
C2	0.20	0.28	0.25	0.60	0.31	0.29	0.32	0.23
C3	0.80	0.32	0.60	0.54	0.72	0.34	0.83	0.21
C4	...	...	0.98	0.19	0.95	0.35	0.85	0.22
C5 (tip)	...	...	1.22	0.59	1.03	0.58	1.19	0.65
Active:								
D1 (base)	...	...	0.48	0.40	0.24	0.35	0.44	0.23
D2	...	...	0.24	0.34	0.21	0.21	0.38	0.21
D3	...	...	0.25	0.14	0.41	0.15	0.74	0.14
D4	...	...	0.49	0.14	0.78	0.18	1.18	0.19
D5 (tip)	...	...	0.97	0.23	1.02	0.00	1.28	0.18
Young:								
E1 (base)	...	...	0.46	0.30	0.40	0.25	0.43	0.25
E2	...	...	0.27	0.25	0.26	0.24	0.19	0.22
E3	...	...	0.85	0.22	0.19	0.19	0.68	0.16
E4 + 5 (tip)	...	...	...	0.15	0.64	0.20	0.97	0.19
Stem:								
Basal (cortex)	0.61	0.49	0.42	0.44	0.52	0.53	0.32	0.18
" (pith)	0.72	0.86	0.63	0.68	0.54	0.58	0.31	0.20
Apical (cortex)	...	0.66	0.76	0.62	0.71	0.50	1.41	0.33
" (pith)	...	0.93	0.70	0.74	0.83	0.68	1.16	0.46

Examination of the data in table IX shows, with certain exceptions, that the amounts of reducing sugars in the sections of the leaves of the exposed shoots were not appreciably greater than those of the covered shoots. The amounts of reducing sugars in the cortex of both the apical and basal sec-

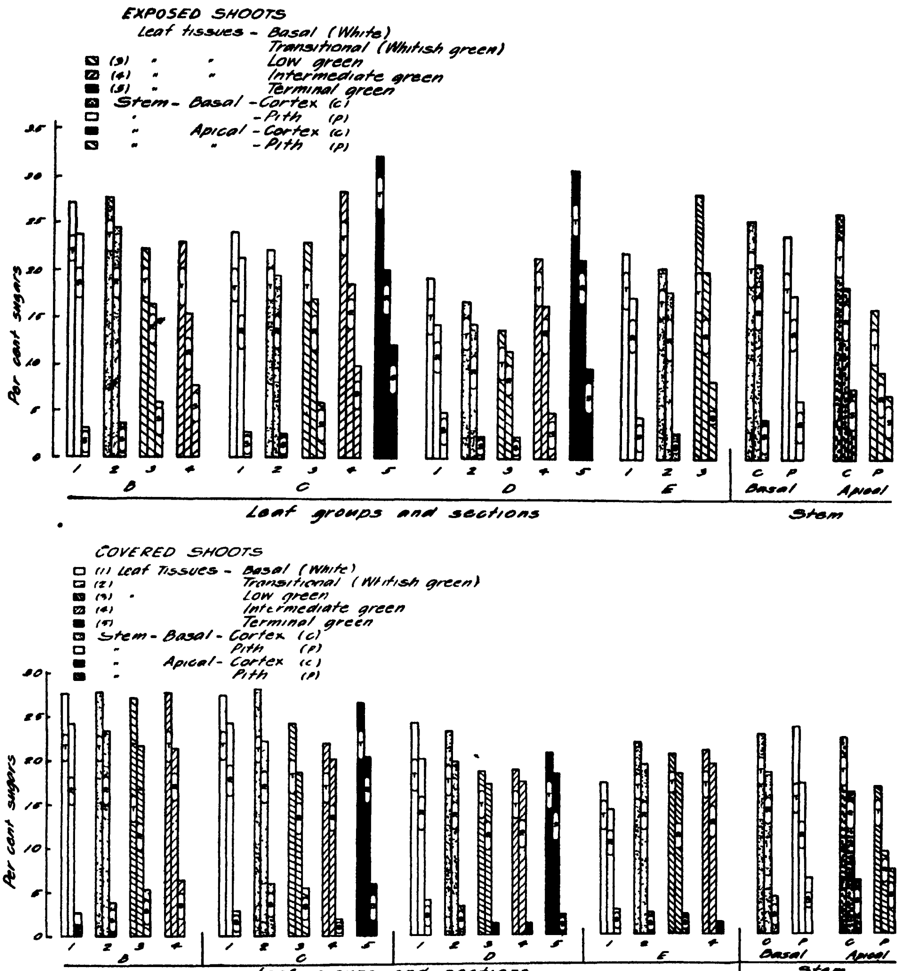


FIG. 9. Percentage of sucrose, reducing, and total sugars on a fresh weight basis in different sections of the leaves and stem of exposed (A) and covered (B) pineapple shoots at the end of the 56 day period.

tions of the stem were greater for the exposed than for the covered shoots. The distribution of the quantities of these sugars in the pith was very irregular. This may be explained on the supposition that reducing sugars are readily converted in the pith—a storage organ—into either sucrose or starch, the two latter being more suitable as reserve substances than the former.

The data on the distribution of sucrose in comparable sections of the leaves and stem of the two lots of shoots show, according to the values in

table X, greater amounts for the exposed than for the covered shoots. There are a few exceptions, occurring mostly in the basal non-chlorophyllous sections of the leaves and in the basal section of the stem, which cannot be explained satisfactorily. The greater amounts of sucrose in the chlorophyllous sections (nos. 3, 4, and 5) of practically all the groups of leaves of the exposed shoots constitute the only positive evidence of sugar accumulations under conditions favorable for photosynthesis. Also, the greater plant weights and greater accumulations of proteins in the sections of the leaves of the exposed than in the sections of the leaves of the covered shoots, produced at the expense of leaf sugars or of other carbohydrates, constitute additional evidence of greater carbohydrate synthesis in the former than in the latter shoots.

Calcium

Calcium was determined for purposes of comparison with nitrate, since the bulk of the nitrogenous salts were supplied to the experimental mother plants as $Ca(NO_3)_2$. Analytical data for the shoots removed at the end of the 105-day period are presented in table XI. They show, in general, that calcium was absorbed in considerably greater quantities by the exposed than by the covered shoots. These quantities were from 1.18 to 12.33 times greater in the various sections of the leaves of the exposed than of the covered shoots. The stem contained greater amounts of calcium than the leaves. Most of the calcium in the stem was confined to the pith, which contained approximately from 1.4 to 2.0 times more calcium than the cortex. The amounts of calcium in the stem sections of the exposed shoots were from 1.19 to 1.45 times greater than in the covered shoots. The data indicate generally that the movement of calcium from the mother plant to the stem of the covered shoots was not as much interrupted as its translocation from pith to cortex and then to leaves. The quantities of calcium in the leaves increased from the transitional (no. 2) to the terminal (no. 5) sections, being in many cases slightly higher in the basal non-chlorophyllous (no. 1) than in the transitional (no. 2) sections. The sections of leaves of chronologically and physiologically advanced groups, excepting the old and senile B group of the exposed shoots, contained, in general, more calcium than those of the less mature and young leaf groups. The distribution of calcium in the different leaf groups of the covered shoots did not vary as much as it did in those of the exposed shoots.

Discussion

The experimental data presented indicate that the growth and physiological behavior of two sister shoots, one exposed to light and the other kept in darkness while attached to their mother plant, were influenced considerably by these conditions.

TABLE XI

MILLIGRAMS OF CALCIUM PER GRAM OF FRESH WEIGHT IN VARIOUS SECTIONS OF THE LEAVES AND STEM OF EXPOSED AND COVERED PINEAPPLE SHOOTS ATTACHED TO THEIR MOTHER PLANT FOR 105 DAYS

PLANT SECTIONS	EXPOSED SHOOTS	COVERED SHOOTS	RATIO: EXPOSED / COVERED
Leaves:			
Old:	*mg.*	*mg.*	
B1 (base)	2.000	1.700	1.18
B2	2.200	0.900	2.45
B3	2.900	0.600	4.82
B4 (tip)	5.200		
Mature:			
C1 (base)	2.700	0.600	4.50
C2	2.000	0.400	5.00
C3	3.700	0.300	12.33
C4	4.800	0.400	12.00
C5 (tip)	7.800	0.800	9.75
Active:			
D1 (base)	1.900	0.400	4.75
D2	1.500	0.500	3 00
D3	1.700	0.500	3.40
D4	2.900	0.800	3.63
D5 (tip)	5.400	1.000	5.40
Young:			
E1 (base)	1.000	0.500	2.00
E2	1 200	0.600	2.00
E3 + 4	1.600	0.600	2.67
E4 + 5 (tip)	3.400	0.800	4.25
Stem:			
Basal (cortex)	5.800	4 000	1.45
" (pith)	9.500	8.200	1.19
Apical (cortex)	7.400	5.600	1.32
" (pith)	11.340	8.000	1.42

Judging from the behavior of the exposed and covered shoots, one may safely state that the chlorophyllous leaf tissues of the covered shoots suffered from serious physiological injury because formation of new chlorophyll was inhibited and that already present underwent some decomposition. With the onset of this latter condition, especially in the terminal sections of aged leaves, a gradual dehydration and death of the tissues involved followed. Any movement of organic substances from the mother plant to the shoots possibly aided only the growth and maintenance of the tissues of the non-chlorophyllous sections of the leaves and the meristematic ones of the stem, but not the highly differentiated chlorophyllous tissues of the leaves.

The shoots kept in darkness were more succulent, that is, they contained greater amounts of water per unit of fresh tissues, because they were unable to increase total organic matter as well as the solute content of their sap with the cessation of photosynthesis.

The data on the distribution of calcium, nitrate, titratable acidity and soluble organic nitrogen are in harmony with those of electrical resistance. The values of electrical resistance for the sections of the covered shoots were, with a few exceptions, higher than those for comparable sections of the exposed shoots, indicating smaller amounts of electrical-conducting solutes in the sap of the former shoots.

The chlorophyllous sections (nos. 3, 4, and 5) of the leaves of exposed shoots contained approximately from 2 to 10 times greater amounts of titratable acidity than comparable sections of the leaves of the covered shoots. The extremely small amounts of titratable acidity in covered shoots may be attributed to cessation of organic acid synthesis in the absence of light and to the utilization of the previously produced and reserve organic acids in the tissues by the protoplasm either as a source for energy or for other metabolic processes. The data suggest that there are possibly two distinct types of physiological processes for the generation of organic acids in the non-chlorophyllous and in the chlorophyllous tissues of pineapple leaves and of the stem. It appears that the processes involved in the generation of organic acids in the chlorophyllous tissues of the leaves are closely associated with those of photosynthesis because they are favored in the presence and are inhibited in the absence of light or possibly of carbohydrates. Whereas, the processes which favor the formation of organic acids in the stem and in the non-chlorophyllous tissues of the leaves appear to be of a respiratory nature, possibly resulting from the incomplete oxidation of carbohydrates. SPOEHR (25) has reviewed the literature on organic acid synthesis and mentions briefly in his book (chapter 5) the theory of Liebig, who considered organic acids as intermediate products in the synthesis of carbohydrates, and that of ROCHLEDER, who assumed that carbon dioxide was reduced to carbon monoxide and the latter was converted to formaldehyde and then to formic acid, which, uniting with carbon dioxide, formed oxalic acid. RUHLAND and WOLF (20) have concluded their review of this subject as follows "... malic acid metabolism is probably linked with that of carbohydrates, either in the process of respiration or of photosynthesis." BENNET-CLARK (2, 3) has proposed, in his extensive review and critique of the literature on the rôle of organic acids in the metabolism of plants, a reconsideration of Baeyer's hypothesis, in favor of that of Liebig, for a more rational interpretation of the mechanism of photosynthesis in view of recent studies (2) which have suggested that malic acid is converted to carbohydrates and that different concentrations found at different times of day are due, in part, to temperature differences and possibly also to different oxygen tensions in the tissues. VICKERY *et al.* (33) have followed the fate of malic acid in excised tobacco leaves kept in nutrient solutions and in darkness for 159 to 279 hours, and state that the amounts of malic acid which disappeared

were greater than those of asparagine found in the tissue, and also that the total acidity did not change significantly. They also observed that the amounts of citric acid increased almost in direct proportion with the amounts of malic acid which had disappeared, this suggesting transformation of malic to citric acid. Our findings suggest that an entirely different kind of mechanism for the disappearance of organic acids operated in pineapple leaves than that observed by VICKERY *et al.* (**33**) in tobacco leaves because the quantities of acids in pineapple leaves decreased in practically all cases.

The data suggest, in general, that the organic acid reserves in the chlorophyllous sections of the leaves may act, possibly after decarboxylation, as sources of energy in the metabolism of pineapple plants.

The amounts of nitrate in the stem and in the non-chlorophyllous tissues were comparatively very high, indicating that assimilation of nitrate in these sections was either very slight or probably non-existent. The etiolated chlorophyllous sections of the leaves of the covered shoots contained comparatively large amounts of nitrate, indicating that the rate of nitrate assimilation had decreased considerably in the absence of chlorophyll and light and possibly because of a lack of sufficient amounts of carbohydrates. The influence of light and of chlorophyll and carbohydrate content on the assimilation of nitrate in the chlorophyllous sections of leaves cannot be segregated in physiological investigations in determining the specific agent or agents for the activation of the mechanism of nitrate assimilation. ANDERSON (**1**) observed that the amounts of nitrate in the etiolated leaves were higher than those found in the green leaves of wheat, oats, and cabbage. These findings are in harmony with ours. SCHIMPER, according to MILLER (**13**) found that nitrates became depleted in green leaves exposed to daylight but that they did not do so in leaves kept in the dark; also that nitrates disappeared in green but not in etiolated leaves. WEBSTER (**34**) found that the roots and stem of soy-bean plants contained, at about the time the seeds matured, greater amounts of nitrate than the leaves. The literature, as reviewed in the first paper of this series (**21**), shows that nitrate is reduced in different plants in light as well as in darkness, and in the presence as well as in the absence of chlorophyll. These generalizations, however, may not hold for all organs of all plants. Indeed, our evidence from the pineapple plant suggests that in the roots, stem, and non-chlorophyllous sections of leaves of pineapple plants nitrate is assimilated either very slowly or probably not at all, while in the chlorophyllous sections of the leaves (with the exception of etiolated leaves kept in the dark) nitrate assimilation is very rapid. The set of conditions essential for the assimilation of nitrate by pineapple plants may differ considerably from that for other plants belonging to different orders and families. If this point of view, *i.e.*, that the set of conditions necessary for the assimilation of nitrate is different for different plants, is

taken into consideration, it may help us in finding order in the chaos of contradictory evidence.

The distribution of reducing sugars in the various sections of the leaves and stem of the exposed and covered shoots show, as may be seen in table IX, that reducing sugars were present in greater quantities in the cortex of the exposed than in that of the covered shoots, while the values for all other sections were inconsistent. With respect to sucrose distribution, the values in most plant sections were higher for the exposed than for the covered plants. These results are in agreement with those of STANESCU (26) who observed that in the leaves of *Acer negundo* and *Medicago sativa* the amounts of monosaccharides remained practically unchanged during both day and night, while the amounts of disaccharides diminished appreciably at night and showed a marked increase during the day. Variations in the sugar content of different plant sections possibly indicate either a high or a low rate of anabolic or catabolic processes, or some specialization in the physiological function of the section or organ. The variation in the content of sucrose or of other substances in different sections may be interpreted if one takes into consideration the photosynthetic ability of different leaf groups, the physiological functions of different sections and the growth activities of the plant at its different stages of development. For instance, sucrose was found in greater quantities in the terminal sections (nos. 4 and 5) of the mature C and fully expanded D group of leaves of the exposed shoots than in the E group of leaves, because of rapid sucrose utilization in the rapidly growing basal tissues of the latter group of leaves. The greater amounts of sucrose in the C and D groups of leaves and smaller amounts in the B group may be attributed to greater protoplasmic vigor and photosynthetic activity in the former than in the latter leaves. PARKIN (16) observed in *Galanthus nivalis* that the sugar content of the leaf increased from the terminal to the basal sections, while the ratio of sucrose to hexose diminished in the same direction. This corresponds approximately with the results obtained for pineapple leaves.

The distribution of calcium, as presented in table XI, shows that this substance was absorbed in greater amounts by the exposed than by the covered shoots. The amounts of calcium and of nitrate in the non-chlorophyllous sections of the leaves and stem of both exposed and covered shoots ran parallel in all groups of leaves, suggesting that both substances were absorbed at approximately equal rates. In the chlorophyllous sections of the leaves no comparison can be made between calcium and nitrate content, owing to the disappearance of the latter through assimilation. No claim is made that calcium and nitrate were absorbed in equimolecular proportions, because other unpublished data have clearly demonstrated that nitrate, under certain conditions, is absorbed at a greater rate than calcium. The

fact that calcium accumulates in the apical section of the stem is of great interest. Because of probable incomplete development of conducting vessels in this meristematic tissue, as is true in other plants, movement of solutes is by diffusion only and is, therefore, slow so that accumulation results. STEWART and ARTHUR (28) have observed that decreased light intensity during the summer increased the dry weight, ash, and phosphorus, but decreased the calcium content in *Lycopersicum esculentum*. HIBBARD and GRIGSBY (9) also found that potassium and calcium accumulated more rapidly in light than in darkness. FREELAND (8) observed that plants with a high transpiration rate, to which category our exposed shoots would belong, had higher values for dry weight, ash, calcium, phosphorus and potassium contents than plants with a low transpiration rate.

It is also of interest to note that the amounts of organic acids in the leaf tissues, reported as titratable acidity, correlated with those of calcium, as found by CLARK (6) for the tomato plant and VICKERY *et al.* (33) for the tobacco plant.

Summary

Forty pairs of sister shoots, one pair per mother plant, were selected in the period from July 20 to 23, 1934, for uniformity and vigor after the ripe fruits of the mother plants had been removed. One of the shoots from each pair was covered with a bag made of green oil cloth which fitted very loosely around the shoot; the other was left exposed. Both covered and exposed sister shoots were left attached to their mother plants for periods of 17, 56, 77, and 105 days, respectively. Between 5 and 10 pairs of sister shoots, representing equal numbers of covered and exposed shoots, were harvested at the end of each period and were studied for growth, distribution of moisture, chlorophyll, electrical resistance, acidity, calcium, and nitrogen and sugar fractions in different sections of the leaves and stem. The results were as follows:

1. The weights of the covered shoots were less at the end of the 77- and 105-day periods than those of the exposed shoots, indicating retardation but not necessarily complete cessation of growth.

2. The moisture content of the various sections of the leaves and stem was consistently greater for the covered than for the exposed shoots, indicating that the total solids, including organic and inorganic substances, per unit of fresh tissues, were greater for the exposed than for the covered shoots.

3. There was a breaking down of chlorophyll in the leaves of covered shoots which had developed before covering and chlorophyll did not form in the leaves that were produced after covering. The chlorophyll content of the exposed shoots varied from one period to another.

4. The electrical resistance of the extracted sap, reported in ohms, was greater for the covered than for the exposed shoots, indicating a greater

content of electrolyte solutes in the sap for the exposed than for the covered shoots.

5. Titratable acidity, reported as percentage of citric acid, was greater in the chlorophyllous sections of the exposed than of the covered shoots. In the non-chlorophyllous sections of the leaves and in the stem, these values were higher for the covered than for the exposed shoots. The results indicate that the titratable acidity content of the chlorophyllous sections of the covered shoots did not increase during the period in which they were covered, but decreased instead, probably as a result of an inadequate carbohydrate supply. The smaller amounts of organic acids in the chlorophyllous sections of the covered shoots were attributed to decarboxylation and the utilization of the by-products by the protoplasm, either as a source of energy or for the synthesis of more complex compounds. The production of organic acids in the chlorophyllous sections of the leaves appeared to be associated either directly or indirectly with photosynthetic processes, since it was favored by light and inhibited by darkness. The generation of organic acids in the non-chlorophyllous tissues of the stem and leaves is of a respiratory type, because organic acid formation was favored by darkness.

6. Nitrate nitrogen was more abundant in the non-chlorophyllous sections of the leaves and in the stem of the exposed than in those of the covered shoots, indicating a higher rate of absorption in the former than in the latter shoots. Values for total organic nitrogen were generally higher for the exposed than for the covered shoots, which is in harmony with the data on nitrate absorption. The relative rate of nitrate assimilation decreased considerably in the chlorophyllous sections of the leaves of the covered shoots, as indicated by the amounts of unassimilated nitrate; this may also account for the lower values of organic nitrogen in the covered than in the exposed shoots.

7. There was no outstanding difference in the amounts of reducing sugars found in the exposed and in the covered shoots, which indicates that these substances were either translocated to other parts of the plant or were constantly regenerated from other more complex compounds stored in the tissues. Sucrose was consistently present in greater amounts in the tissues of the exposed than in those of the covered shoots. Variations in the sucrose content of different leaf and stem sections are discussed.

8. Calcium was found in greater quantities in the leaf and stem sections of exposed than of covered shoots, indicating that absorption was greater by the former than by the latter shoots. The amounts of calcium were greater in the pith of the apical section of the stem than in any other section. It is suggested that these large amounts of calcium in the meristematic tissues of the stem may be the result of accumulation due to incomplete development of conducting vessels.

9. The data show, in general, that sister shoots receiving a great amount of their inorganic nutrition and water supply by way of the roots and stem of the mother plant differ considerably in their physiological behavior and chemical composition when one is exposed to light and the other is kept in darkness.

LITERATURE CITED

1. ANDERSON, V. L. Some observations on the nitrate reducing properties of plants. Ann. Bot. **38**: 699–706. 1924.
2. BENNET-CLARK, T. A. The rôle of the organic acids in plant metabolism. New Phytol. **32**: 37–71; 128–161; 197–230. 1933.
3. ——————. Organic acids in plants. Ann. Rev. Biochem. **6**: 579–594. 1937.
4. BROWN, W. H., and TRELEASE, S. F. Alternate shrinkage and elongation of growing stems of *Cestrum nocturnum*. Philippine Jour. Sci. C. **13**: 353–360. 1918.
5. BURKHOLDER, P. R. The rôle of light in the life of plants. Bot. Rev. **2**: 1–52; 97–172. 1936.
6. CLARK, H. E. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**: 5–24. 1936.
7. EISENMENGER, W. S. The distribution of nitrogen in tobacco when the supplies of nitrogen and of light are varied during the growing period. Jour. Agr. Res. **46**: 255–265. 1933.
8. FREELAND, R. O. Effect of transpiration upon the absorption and distribution of mineral salts in plants. Doctor's Dissertation, no. 17. The Ohio State University Press. 1935.
9. HIBBARD, R. P., and GRIGSBY, B. H. Relation of light, potassium, and and calcium deficiencies to photosynthesis, protein synthesis translocation. Michigan Agr. Exp. Sta. Tech. Bull. 141. 1934.
10. IVANOFF, M. Versuche über die Frage, ob in den Pflanzen bei Lichtabschluss Eiweisstoff sich bilden. Land. Vers.-Sta. **55**: 78–94. 1901.
11. KERTESZ, Z. I. Recalculated tables for the determination of reducing sugars by Bertrand's method. B. Westermann Co., New York. 1930.
12. KISHI, Y., and YOKOTA, Y. Studies in the change of chemical constituents of mulberry leaves in the intercepted sunlight. (English Sum.) Bull. Sci. Fak. Terkult Kyusu Imper. Univ. **6**: 103–104. 1935.
13. MILLER, E. C. Plant physiology. McGraw-Hill Book Co., New York. 1931.

14. Nightingale, G. T., and Schermerhorn, L. G. Nitrate utilization by asparagus in the absence of light. Science n.s. **64**: 282. 1926.

15. ———, ———, and Robbins, W. R. The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems and leaves. New Jersey Agr. Exp. Sta. Bull. **461**: 1–38. 1928.

16. Parkin, J. The carbohydrates of the foliage leaf of the snow drop (*Galanthus nivalis*) and their bearing on the first sugar of photosynthesis. Biochem. Jour. **6**: 1–47. 1912.

17. Priestley, J. H. Light and growth. III. An interpretation of phototropic growth curvatures. New Phytol. **25**: 213–247. 1926.

18. ———, and Ewing, J. Physiological studies in plant anatomy. VI. Etiolation. New Phytol. **22**: 30–44. 1923.

19. Richards, H. M. Acidity and gas interchange in cacti. Carnegie Inst. Washington, Pub. no. 209. 1915.

20. Ruhland, Wilhelm, and Wolf, J. Metabolism of carbohydrates and organic acids in plants. Ann. Rev. Biochem. **5**: 485–512. 1936.

21. Sideris, C. P., Krauss, B. H., and Young, H. Y. Assimilation of ammonium and nitrate nitrogen from solution cultures by roots of *Pandanus veitchii* Hort. and distribution of the various nitrogen fractions and sugars in the stele and cortex. Plant Physiol. **12**: 899–928. 1937.

22. ———. Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. **13**: 489–527. 1938.

23. ———. Distribution of different nitrogen fractions, sugars and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts. Plant Physiol. **14**: 227–254. 1939.

24. ———. Distribution of nitrogen fractions, sugars, and other substances in *Ananas*, grown in darkness versus daylight. Plant Physiol. **14**: 647–676. 1939.

25. Spoehr, H. A. Photosynthesis. Chemical Catalogue Co., New York. 1926.

26. Stanescu, P. P. Les variations quantitatives des produits de la photosynthése dans les feuilles des plantes vertes pendant un jour (24 heures). Thèse, Université de Bucarest, 1927.

27. Street, O. E. Carbohydrate-nitrogen and base element relationships of peas grown in water culture under various light exposures. Plant Physiol. **9**: 301–322. 1934.

Stewart, W. D., and Arthur, John M. Change in mineral composition of the tomato plant irradiated with a quartz-mercury vapor

lamp and its relation to the level and ratio of calcium and phosphorus in the nutritive medium. Contrib. Boyce Thompson Inst. **9**: 105–120. 1937.

29. Tolmachev, Ivan. Effect of darkness and light on the organic acids in the plant. Lapiski Kiiv. Sil's Ko-Gospod. Inst. (Kyiv. Agr. Inst. Mem.) **2**: 1–21. 1927.
30. Tottingham, W. E., and Lease, E. J. A photochemical aspect of nitrate assimilation in plants. Science n.s. **80**: 615–616. 1934.
31. Trelease, S. F. Night and day rates of elongation of banana leaves. Philippine Jour. Sci. **23**: 85–96. 1923.
32. Vickery, H. B., Pucher, G. W., Leavenworth, C. S., and Wakeman, A. J. Chemical investigations of the tobacco plant. V. Chemical changes that occur during growth. Connecticut Agr. Exp. Sta. Bull. **374**: 553–619. 1935.
33. ———, ———, ———, and ———. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in leaves during culture in light and darkness. Connecticut Agr. Exp. Sta. Bull. **399**: 753–832. 1937.
34. Webster, J. E. Nitrogen metabolism in the soy bean. Plant Physiol. **3**: 31–43. 1928.
35. Wolf, Johannes. Beitrag zur Kenntnis des Saürestoffwechsels succulenter Crassulacaen. Planta **15**: 572–644. 1931.

RESPIRATION OF CEREAL GRAINS AND FLAXSEED[1]

C. H. BAILEY

(WITH FIVE FIGURES)

Introduction

In earlier papers by BAILEY (2, 3) and BAILEY and GURJAR (4, 5a, 5b, 5c, 5d), the researches in this field prior to 1921 were reviewed, and the relationship of moisture content, damaged and broken kernels, and various other factors to the relative level of respiration of wheat, rice, and corn (maize) was traced. These laboratory investigations paralleled or supplemented certain large scale, or practical, studies of grain storage. It became evident that the prediction of the behavior of stored grain as determined by chemical composition, physical characteristics, and the several biological factors involved in keeping qualities, is not a simple matter. The weighting of these several variables, and the extent to which they are related to factors of environment, including climatic conditions, composition of the air in the grain bin, and ventilation becomes very involved. Despite these practical difficulties, it still appears that the laboratory attack upon certain of the details of this problem is a logical procedure and will ultimately lead to the most satisfactory solutions.

Observations made during commercial operations of grain handling and storage, while interesting and useful as respects the individual parcels of grain involved, are of limited value in effecting an analysis of the several factors operative in such instances. Thus there is a definite lack of control of many variables in these cases, certain of which cannot even be held constant during any extended period. Moreover, there is also the formidable item of expense when the storage of thousands of bushels of grain is involved, which places such work outside of the reach of modest research budgets.

Since the publication of the papers cited above, several contributions to the literature have appeared, and certain of these tie into the researches reported in this paper so definitely as to necessitate a brief review of them before detailing the data accumulated by the author. COLEMAN, ROTHGEB, and FELLOWS (10) observed much the same relationships between moisture content, and other factors of quality of sorghum, and the rate or respiration as measured in terms of CO_2 produced per unit of time and material, that had previously been reported for corn (maize) by BAILEY (2). The actual levels of respiration were lower in sorghum than in maize, however.

BAKKE and NOECKER (6) studied the relation of moisture to respiration

[1] These studies were made possible by a grant from the Research Funds of the Graduate School of the University of Minnesota.

and heating in stored oats. They concluded that heating is negligible when the moisture is below 15.0 pèr cent. The same rapid increase in rate of respiration as the moisture increased was observed in this study as had previously been found to be true of corn, wheat, and sorghums. The high temperatures developed in moist grain during self-heating was found to lower the vitality of the seed. These authors attributed temperature variations of various samples at the same moisture level to variations in the fungal flora of different seed lots.

BROWNE (7) gives a good historical review of the early observations of the spontaneous heating of agricultural products and a critical discussion of past and present theories concerning the phenomenon. He points out that although heating is a common occurrence, the exact coincidence of all the conditions necessary for the spontaneous ignition of agricultural products is fortunately infrequent. He indicates that more information is needed on these subjects, such as the elementary analysis of the heating material, analysis of the solid, liquid and gaseous organic compounds that are produced in both anaerobic and aerobic fermentation, and knowledge of moisture migration and heat transference.

AGRONOMOFF (1) engaged in an extensive study of sound, dry cereal grains stored in large elevator bins. He observed that the thermal conduction of such material is low. The elevation of temperature in the summer is accordingly a function of the thickness of the layer where the grain is not disturbed. In the instance of moist oats containing 14.8 per cent. water, there was a substantial increase in temperature during the summer as contrasted with dry oats containing 11.0 per cent. water. Moist grain during the summer should not be placed in outside or corner silos, he concluded. He also comments on the increased development of granary weevils in consequence of the elevation of temperature in spring and summer.

GILMAN and BARRON (12) conclude that the temperatures reached in a bin of stored grain are due to the interaction of several factors. These include the "heat of germination" itself and temperatures generated by the growth of molds developing on the grain saprophytically. JAMES, RETTGER, and THOM (16) as part of their study of microbial thermogenesis had previously discussed the production of heat which accompanies the growth of micro-organisms, contending that in the presence of air or oxygen micro-organisms are capable of producing comparatively high temperatures. The fact that molds may effect an elevation of temperature when grown on suitable organic substances had been shown repeatedly prior to this study.

GILMAN and BARRON (12) undertook their investigation with the object of isolating and evaluating the rôle of each of these two sources of heat in the temperature increase occurring in stored grains. They compare the temperatures reached when sterilized oats, wheat, and barley at 18 to 30 per

cent. moisture levels are stored in Dewar flasks. The seeds were inoculated with three species of Aspergillus while uninoculated seeds served as controls. In these experiments the temperature of the check samples remained approximately the same during a six-day period while the samples with fungal growth rose rapidly from a temperature of 30° to 50° C. In this connection WILSON (22) has suggested that a temperature of 30° C. affects the protoplast of the kernel in a manner which permits the outward leaching of soluble food materials that are readily attacked by the fungi commonly present.

SWANSON (21) states that a relatively high moisture content will accelerate respiration in the grain and favors the growth of molds, both of which will generate heat. The percentage of moisture at which molds developed was closely related to the temperature, since there was no mold growth on wheat stored at 60° F. even though the moisture content of the wheat was 18 per cent. Mold growth is considered an indication of damage in stored grain.

ROZSA (19) discussed PAPS's investigation which indicated that moldiness did not become a prominent factor in wheat spoilage until the relative humidity of atmospheres in hygroscopic equilibrium with the grain exceeded 75 per cent. This level of humidity is in equilibrium with wheat containing about 15 per cent. of moisture according to the average of PAPS's data, which does not agree with that of COLEMAN and FELLOWS (9), since the latter found that 17 to 17.5 per cent. of moisture was in equilibrium with a 75 per cent. relative humidity. Moreover, SKOVHOLT and BAILEY (20) found that common molds, including *Penicillium expanium* and *Rhizopus nigricans,* were very sensitive to humidity levels and failed to grow on bread crust until the humidity was in excess of 85 per cent. and the moisture content of the material about 20 per cent. *Aspergillus niger* grew at a slightly lower level of humidity but only to a very slight degree at 85 per cent. humidity. Incidentally, a humidity of 85 per cent. was found by COLEMAN and FELLOWS (9) to be in equilibrium with wheat having a moisture content of about 21.5 per cent. although PAPS's findings placed the equilibrium point at about 18 per cent. moisture.

Even though these several studies do not entirely agree, it does appear that an acceleration of respiration of bulk wheat in consequence of mold growth is not likely to occur until the moisture content of the grain exceeds that which is safe for ordinary storage. In the writer's judgment molds will not proliferate freely until the moisture content of wheat is in excess of 17.5 per cent. moisture, which is substantially above the level permitted under the U. S. Grain Standards for the grades commonly accepted for delivery under contract.

LARMORE, CLAYTON, and WRENSHALL (14) studied the respiration of

hard spring wheat. They realized that the true respiration of wheat is complicated by the respiration of fungi which develops on damp wheat. In this study the germination and growth of fungi was controlled effectively by toluene and carbon tetrachloride vapors. In the presence of these substances carbon dioxide was produced at a low rate and no heating occurred in wheat of 25 per cent. moisture content.

Methods

Samples of grain to be tested were brought to approximately 11, 13, 15, and 17 per cent. moisture levels by the addition of water to weighed portions of dry (11 per cent. moisture or less) grain contained in large, tightly-stoppered bottles. While moisture equilibrium was being attained the samples were stored at 2° C. to restrain the growth of micro-organisms until after the surface water had been absorbed by the interior tissues of the kernels. After three or four days of such storage the samples were brought to room temperature and thoroughly mixed, by using a grain divider, and the desired quantity (from 250 to 500 grams, depending upon the moisture content) of the grain was weighed into the respiration chambers.

The moisture content of the sample was determined, at the same time, by weighing out into Petri dishes duplicate 20-gram portions of grain. These samples were allowed to air dry in the laboratory and the loss in weight was noted carefully. The air-dried samples were then ground in a burr mill and two-gram portions weighed up for the final determination of moisture by the Association of Official Agricultural Chemists (1937) air oven method. This double moisture determination, while time consuming, is necessary if reliable moisture determinations are to be obtained on wetted grain.

Several duplicate grain samples were placed in sheet-metal towers fitted with an inlet and outlet tube and otherwise tightly sealed. All rubber connections were thoroughly paraffined since carbon dioxide may diffuse through rubber not protected in some such manner. The metal grain cylinders were placed in an air thermostat maintained at 37.8° C. (100° F.) which is the temperature at which the earlier investigations were conducted by BAILEY *et al.*

The essential features of the apparatus for the absorption of carbon dioxide respired by grain are shown in figure 1. Entering air was passed through a gas meter in order to determine the total volume of gas displaced in the system. In all determinations sufficient air was admitted to replace four times the total volume of gas in the entire system. The incoming air was freed from carbon dioxide by conducting it through a large soda lime tower. This CO_2-free air was then passed through a series of bottles containing sulphuric acid of the proper specific gravity to bring the escaping air to a relative humidity in equilibrium with the hygroscopic moisture content of the grain to be aspirated in order to avoid moisture changes in the

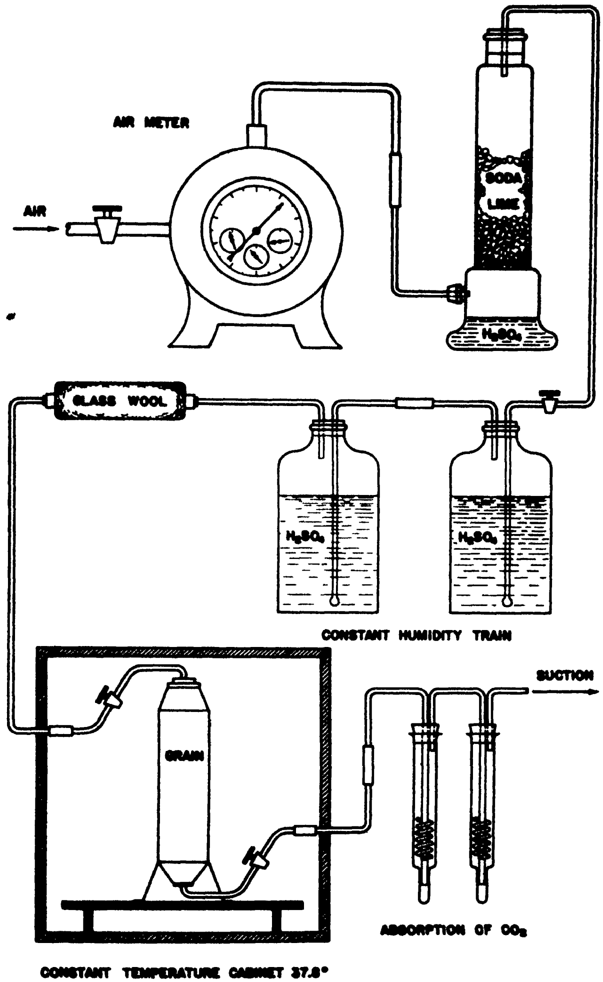


FIG. 1. Diagram of apparatus used in determining the rate of respiration of grain.

grain during the aspiration process. The data for adjusting atmospheres to the appropriate relative humidity were taken from the papers by COLEMAN and FELLOWS (9), and WILSON (22).

The humidified CO_2-free air forced the accumulated CO_2 from the respiration chamber into a measured quantity of standard barium hydroxide solution contained in specially constructed absorption vessels as indicated in the accompanying diagram. The mixture of air and respired CO_2 was forced down the central tube whence it rose slowly in small bubbles through the spiral tube. The barium carbonate precipitate fell to the bottom of the absorption tube and did not obstruct the continued passage of gas through

the solution. By regulating the flow of gas to a slow, steady stream a very effective absorption was attained. When heavy concentrations of carbon dioxide were encountered two or more units were connected together thus assuring that no appreciable carbon dioxide gas escaped absorption. An even current of gas through the absorption tubes could be maintained by applying a slight suction at the end of the entire system. This also prevented any rise of pressure in the system which might unseat the stoppers.

Titration of the residual barium hydroxide was carried out directly in the absorption vessel against standard HCl solution with phenolphthalein as an indicator and using the glass spiral as a stirrer. The titration procedure employed was similar to that used by GURJAR (15). MARTIN and GREEN (18) have shown that spiral absorbers are a very effective means for absorbing carbon dioxide and also that titrating directly in the absorption vessel in the presence of suspended barium carbonate does not cause a loss of carbon dioxide through the decomposition of the carbonate by the acid added during the titration.

In the studies previously reported from this laboratory the CO_2 of respiration was allowed to accumulate for four days and was then removed and determined. This total was divided by four to give the average per 24 hours in which form it was recorded. The same procedure was followed in the researches here reported, and in addition a parallel series was provided from which the respired CO_2 was removed at the end of each 24-hour period for four consecutive days and the total divided by four to give the average per 24 hours. Both sets of data are recorded in the following tables. It will be noted that daily aspiration resulted invariably in larger average yields of respired CO_2, but the two sets of data yield curves of the same general shape if corrected for the differences in quantities involved. Accordingly it appears that there is no singular advantage attached to one technique over the other in determining the effect of the several variables studied upon the *relative* level of respiration.

Continuous aspiration was not attempted because of the obvious difficulties involved in preventing changes in the moisture content of the grain in consequence of the continuous passage of a current of air through it.

Experimentation

RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF OATS

Four varieites of clean, sound oats from the 1933 crop grown on the University Farm, St. Paul, Minnesota, were obtained for the determination of respiration rates at various moisture levels. In this study an attempt was made to obtain sufficient data so that the average value reported would give a correct indication of the rate of respiration of the grain under the conditions noted. Thus each value recorded for the four moisture levels

represented the average of at least eight separate determinations. It is obviously impractical to report all of these detailed data. Therefore, in addition to the use of average values for individual samples, further condensation is accomplished by reporting only the average respiratory rate values of several samples.

Table I and figure 2 indicate the average rate of respiration as a function

TABLE I

AVERAGE RATE OF RESPIRATION OF FOUR VARIETIES OF OATS

Carbon dioxide respired per 24 hours per 100 gm. dry matter		
Moisture	Daily aspirations	Aspirated at end of four day period
%	mg.	mg.
11.0	0.2	0.1
13.0	0.7	0.2
15.0	2.8	1.4
17.0	13.5	8.0

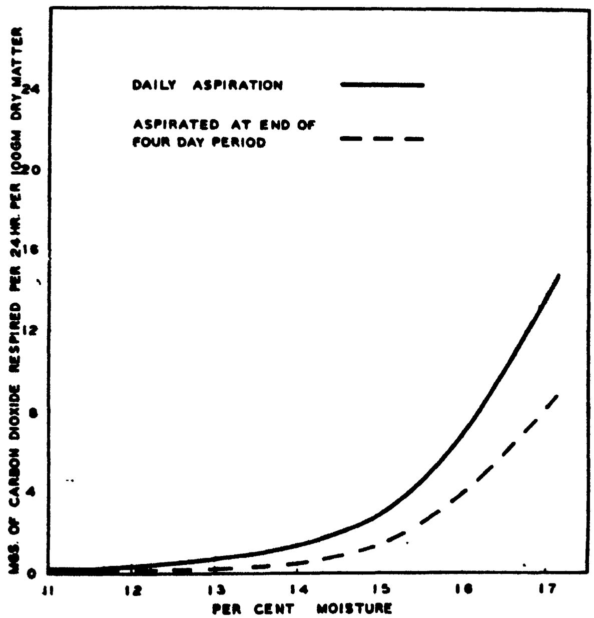


FIG. 2. Respiration of oats showing the retarding influence of accumulated carbon dioxide on respiratory rate.

of moisture content in the instance of three varieties of oats. The values reported in this and subsequent tables were obtained by plotting graphs of the individual respiration curves. By interpolation of these curves carbon

dioxide respired (in milligrams) at the moisture levels of 11, 13, 15 and 17 per cent. was computed. When water was added to the dry samples of grain it was not possible to secure exactly the percentage of moisture desired; it was seldom, however, that the actual moisture deviated from the desired level by more than 0.5 per cent.

Table I and figure 2 present typical examples of the increase in rate of respiration of grain as the moisture content exceeds 15 per cent. Likewise, the retarding influence of accumulated carbon dioxide on the respiratory rate is plainly indicated. LARMORE, CLAYTON, and WRENSHALL (17) also found that the respiration rate in continuously aspirated grain was higher than when discontinuous aspiration was applied. With discontinuous aspiration the rate was dependent upon the free space in the container.

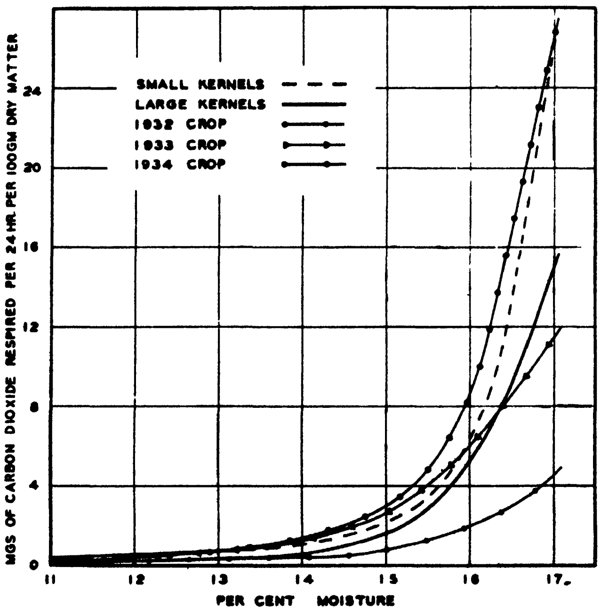


FIG. 3. Respiration of barley showing the influence of kernel size and the differences between crop seasons.

The average respiratory rate of all samples of oats compared with other grains is shown in table IX and figure 4. These values represent the accumulated carbon dioxide after a four-day undisturbed storage period and by computing the rate on the basis of milligrams of carbon dioxide respired per twenty-four hours per one hundred grams of dry matter.

RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF BARLEY

The respiratory rate of barley at various moisture levels was determined in the same manner as that just described for oats. In order to compare,

from year to year, the respiration rate of barley grown in the same locality and on the same soil a further investigation was made by obtaining samples of barley grown at University Farm during the crop seasons of 1932, 1933, and 1934. In table II and figure 3 the data from the three crop years are recorded separately and it appears that the 1933 and 1934 crop samples behaved similarly, but that the respiration of the 1932 crop was considerably lower. This may be attributed in part at least, to the fact that the 1933 and 1934 crop growing periods were extremely dry and, hence, the barley kernels were of smaller size and shriveled, while 1932 was a year of normal rainfall in the locality where the barley was grown which tended to produce more plump kernels. The viability of the barley samples for the three years was nearly the same. The relation between kernel size and rate of respiration will be discussed later on in this paper. It is evident from the data presented in table II that at the same moisture level there is a variation in the likelihood of barley heating during storage, as crop conditions change from year to year.

TABLE II

COMPARISON OF THE RATE OF RESPIRATION OF BARLEY FOR THE YEARS 1932, 1933 AND 1934

MOISTURE	YEAR	CO_2 RESPIRED PER 24 HR. PER 100 GM. DRY MATTER	
		DAILY ASPIRATION	ASPIRATED AFTER 4 DAYS
%		mg.	mg.
11.0	1932	0.1	0.05
11.0	1933	0.1	0.05
11.0	1934	0.3	0.20
13.0	1932	0.3	0.1
13.0	1933	0.7	0.4
13.0	1934	0.7	0.4
15.0	1932	0.8	0.5
15.0	1933	2.6	2.0
15.0	1934	3.0	2.4
17.0	1932	4.9	2.8
17.0	1933	11.6	9.1
17.0	1934	26.4	16.0

The average respiratory rate of all samples of barley is reported in table IX and figure 4. Compared to other grains, barley is intermediate in its rate of respiration.

RELATION BETWEEN MOISTURE CONTENT AND RESPIRATION OF RYE

The rye samples included in this study evidenced a low respiratory rate as indicated by the values recorded in table III and shown graphically in figure 4. There should be a correspondingly reduced hazard of rye heating

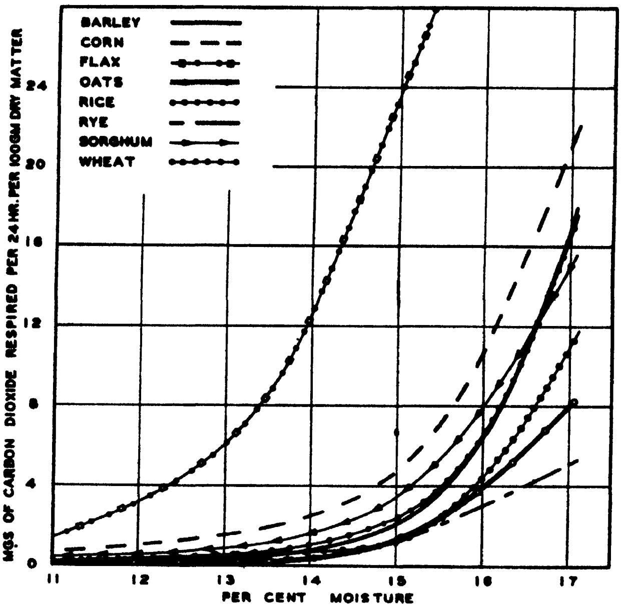


FIG. 4. Comparative respiration of various grains and flax seed.

during storage when harvesting conditions are normal. The small germ area of rye compared with the total area of the kernel, and the relatively smooth surface of the rye kernel, which is not conducive to micro-organism growth may both contribute to the low rate of respiration of this cereal.

RESPIRATION OF FLAXSEED

Table IV and figure 4 indicate that flaxseed has the highest respiratory rate of any seed thus far reported. This is not unexpected in view of the high oil content of flax seed. The known fact that the embryo cells of seeds contain a high proportion of lipids and likewise respire much more vigor-

TABLE III

AVERAGE RATE OF RESPIRATION OF RYE

MOISTURE	CARBON DIOXIDE RESPIRED PER 24 HR. PER 100 GM. DRY MATTER	
	DAILY ASPIRATIONS	ASPIRATED AT END OF 4-DAY PERIOD
	mg.	*mg.*
11.0	0.2	0.1
13.0	0.5	0.3
15.0	2.0	1.2
17.0	9.3	5.1

TABLE IV

AVERAGE RATE OF RESPIRATION OF FLAX

MOISTURE	CARBON DIOXIDE RESPIRED PER 24 HOURS PER 100 GRAMS DRY MATTER	
	DAILY ASPIRATIONS	ASPIRATED AT END OF 4-DAY PERIOD
%	mg.	mg.
11.0	2.5	1.4
13.0	9.2	6.1
15.0	48.4	23.9
17.0	159.3	67.4

ously than endosperm cells would suggest that oil seeds such as flax might be expected to have a higher respiratory level than farinaceous material.

Another significant feature of this situation is the probable distribution of the moisture in an oil seed. When approximately 40 per cent. of the dry substance of a flaxseed is an oil that is essentially immiscible with water, it must follow that the actual moisture content of the hygroscopic substance present will be higher than in a farinaceous endosperm comprised largely of hydrophilic materials. Thus, for example, if we assume that a flaxseed contains 40 per cent. of oil (dry matter basis) that is immiscible with water, then an apparent moisture content of 10 per cent. of the entire seed might mean an actual content of water in the hydrophilic substances of approximately 16.5 per cent. Applying such a correction to the data recorded in table IX and figure 4 would tend to bring the flaxseed values in line with those of the cereal grains. Of course the problem is not as simple as is suggested by such a calculation, but the issue raised here may be significant.

RELATION OF RESPIRATION RATE TO THE GRAIN GRADE

To determine the rate of respiration of samples of barley, oats, and rye such as are actually being handled and stored in the grain trade, composite samples of different grades of these three grains were obtained from the State Grain Inspection Department in Minneapolis. The Federal standards recognize a number of numerical grades of grain based on such factors as: limits of foreign material, test weight, damaged kernels, moisture, etc. Four grades of barley, three grades of oats, and three grades of rye were thus procured. Since the samples were composite ones no basis for the differentiation in grade can be given. In all cases, however, the samples were composed of sound grain.

The results of the study using these samples are recorded in tables V, VI, and VII. All grades show the same rapid rise in production of carbon dioxide when the moisture level is above 15 per cent. There is, moreover,

TABLE V

COMPARISON OF THE RATE OF RESPIRATION OF FOUR GRADES OF BARLEY AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

MOISTURE	GRADE	CO_2 RESPIRED PER 24 HR. PER 100 GM. DRY MATTER	
		DAILY ASPIRATION	ASPIRATED AFTER 4 DAYS
%		*mg.*	*mg.*
11.0	No. 2	0.4	0.2
11.0	No. 3	0.2	0.1
11.0	Sample	0.1	0.1
11.0	Feed	0.2	0.2
13.0	No. 2	0.7	0.4
13.0	No. 3	0.4	0.3
13.0	Sample	0.4	0.2
13.0	Feed	1.0	0.6
15.0	No. 2	2.3	1.8
15.0	No. 3	1.6	1.0
15.0	Sample	1.5	0.8
15.0	Feed	3.0	2.0
17.0	No. 2	12.7	9.4
17.0	No. 3	19.0	13.3
17.0	Sample	8.4	5.4
17.0	Feed	19.9	18.5

TABLE VI

COMPARISON OF THE RATE OF RESPIRATION OF THREE GRADES OF OATS AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

MOISTURE	GRADE	CO_2 RESPIRED PER 24 HR. PER 100 GM. DRY MATTER	
		DAILY ASPIRATION	ASPIRATED AFTER 4 DAYS
%		*mg.*	*mg.*
11.0	No. 3	0.2	0.1
11.0	No. 4	0.2	0.1
11.0	Sample	0.2	0.1
13.0	No. 3	0.4	0.2
13.0	No. 4	0.4	0.2
13.0	Sample	0.5	0.3
15.0	No. 3	0.9	0.6
15.0	No. 4	1.5	0.8
15.0	Sample	2.7	1.8
17.0	No. 3	21.1	8.5
17.0	No. 4	12.6	7.0
17.0	Sample	18.0	7.7

TABLE VII

COMPARISON OF THE RATE OF RESPIRATION OF THREE GRADES OF RYE AT THE SAME MOISTURE LEVEL. INTERPOLATED VALUES

Moisture	Grade	CO_2 respired per 24 hr. per 100 gm. dry matter	
		Daily aspiration	Aspirated after 4 days
%		*mg.*	*mg.*
11.0	No. 1	0.2	0.1
11.0	No. 2	0.1	0.1
11.0	No. 3	0.2	0.1
13.0	No. 1	0.4	0.2
13.0	No. 2	0.4	0.2
13.0	No. 3	0.7	0.4
15.0	No. 1	1.7	1.0
15.0	No. 2	2.6	1.7
15.0	No. 3	2.3	1.3
17.0	No. 1	6.7	5.2
17.0	No. 2	9.9	5.7
17.0	No. 3	14.0	6.1

considerable variation in the quantity of carbon dioxide evolved by the different grades at the same moisture level, but the variation does not appear to be related to the grade.

Relation of the relative plumpness of the barley kernel to the rate of respiration

Two samples of barley which differed greatly in kernel size were obtained from one of the malting plants in Minneapolis. These samples are here designated as large and small size kernels; to the maltster they are known as "malting size" and "needles" respectively. The 1000 kernel weights were 39.2 and 17.2 grams respectively, and germination was 94 per cent. in each case. Table VIII shows the respiration data of these two lots of barley and the same data are shown graphically in figure 3. The decided differences in the rate of respiration of large and small kernels from the same lots of barley substantiates what has previously been contended; namely, that for equal weights of different sized kernels the smaller kernels have the highest respiration rate. Bailey and Gurjar (4) found this to be true of wheat kernels.

Change in the dielectric properties of oats and barley with change in the moisture content of the grain

It is generally recognized that the water present in a hydrogel exists in several states or conditions. As suggested by Foote and Saxton (11) these

TABLE VIII

RATE OF RESPIRATION OF BARLEY

Moisture	Carbon dioxide respired per 24 hr. per 100 gm. dry matter	
	Large size kernels	
	Daily aspiration	Aspirated at end of 4 day period
%	mg.	mg.
11.49	0.26	0.13
13.07	0.40	0.32
15.05	1.68	1.32
17.07	14.99	13.89
	Small size kernels	
11.00	0.21	0.14
12.95	0.71	0.48
15.35	2.95	2.05
17.02	26.40	22.26

states may be described as free, capillary, and combined. GORTNER (14) has ably discussed the free and bound states of water in biological material and the differences in physical properties between these two states. It has recently developed that indirect physical measurements were becoming increasingly useful and significant in distinguishing between these states. One such means involves the observation of changes in capacitance of such materials as cereal grains as a function of the amount, and hence of the state of water present.

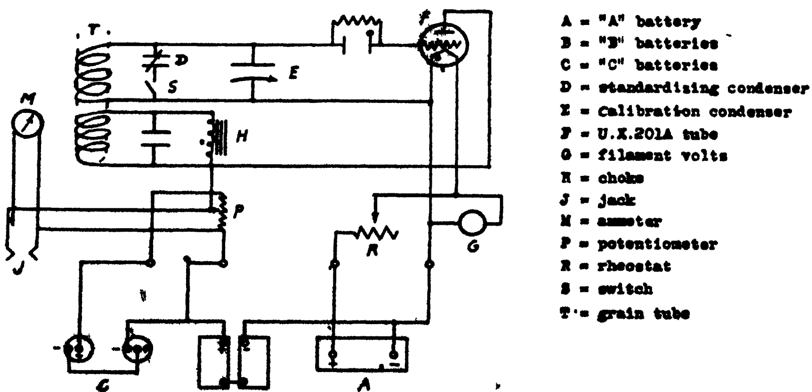


FIG. 5. Diagram of the electrical circuit of the BURTON-PITT apparatus.

The BURTON-PITT (8) device which was available in this laboratory is adapted to this end. It makes use of the high frequency circuit diagrammed

in figure 5. When a tube containing grain is introduced into the electrical field produced by the current at the position marked T in the diagram, a change occurs in the current flowing through the ammeter, which can be observed and recorded.

Observations were accordingly made upon one series each of oats and barley samples, in which the sole variable was moisture content. The rate of respiration, and the milliammeter reading as observed with the BURTON-PITT device are recorded in table X. While these two sets of values are not

TABLE IX

COMPARISON OF THE RATE* OF RESPIRATION OF GRAINS AT FOUR MOISTURE LEVELS. RESULTS EXPRESSED IN MGS. OF CO_2 PER 24 HR. PER 100 GM. DRY MATTER

KIND OF GRAIN	PERCENTAGE MOISTURE			
	11	13	15	17
	mg.	*mg.*	*mg.*	*mg.*
Barley	0.1	0.4	2.1	16.7
Corn†	0.7	1.5	4.7	21.9
Flax	1.4	6.1	23.9	67.4
Oats	0.1	0.2	1.4	8.0
Rice‡	0.2	0.5	2.4	16.5
Rye	0.1	0.3	1.2	5.1
Sorghum§	0.5	0.9	3.5	15.0
Wheat§§	0.2	0.5	1.2	11.0

* Values obtained by interpolation and extrapolation of data.
† Average values from (3).
‡ Values from (5b).
§ Values from (10).
§§ Values from (4).

TABLE X

AVERAGE RATE OF RESPIRATION AND BURTON-PITT MOISTURE VALUES FOR ALL SAMPLES OF OATS AND BARLEY

MOISTURE	OATS		BARLEY	
	CO_2 PER 24 HR. PER 100 GM.	BURTON-PITT VALUES	CO_2 PER 24 HR. PER 100 GM.	BURTON-PITT VALUES
%	*mg.*	$\frac{MA \times 100}{wt.\ of\ grain}$	*mg.*	$\frac{MA \times 100}{wt.\ of\ grain}$
11.0	0.2	0.55	0.2	.49
13.0	0.5	0.63	0.6	.54
15.0	1.9	0.82	1.6	.69
17.0	11.3	1.09	13.7	.84

exactly parallel, they approach that relationship. As indicated by GORTNER (13) the sharp acceleration of respiration at certain levels of moisture content may be the consequence of bound water relationships, and it is also probable that the same is true of the observed capacitance values.

While the data here recorded do not make certain facts abundantly clear, it has appeared from extensive experience with the BURTON-PITT device that the milliammeter readings are a function of grain properties other than moisture content. In fact the same variables which affect respiratory level of grain likewise tend to influence the milliammeter readings in the same direction and to much the same degree. It will require additional research to establish this fact, but it appears to be a safe prediction that such readings as are secured by this instrument will constitute more useful criteria of the keeping qualities of grain in storage than any single chemical or physical characteristic of which we are aware.

The exponential types of curve which expresses the relation between the moisture content of corn and respiratory rate suggests that log (CO_2 respired) might become a linear function of moisture content. Letting $y =$ CO_2 respired per 100 gm. dry matter in each 24 hours, and $x =$ moisture content, log y was plotted against x and this turned out to be a curve rather than a straight line. This made it evident that the values for y must be corrected, possibly by a constant c. The value for c was computed through the use of the equation $y = ae^{bx} + c$. and was found to be $c = 0.672$. Accordingly when all values for y were corrected by subtracting 0.672 and log (y–c) was plotted against x a straight line resulted. For average corn of all varieties each increase in moisture content of 1 per cent. was accompanied by an increase in the value of log $(y - 0.672) = 0.350$. In a similar calculation applied to the data resulting from the study of sound hard spring wheat $c =$ 0.4926 and the average increase in the value log $(y - 0.4926) = 0.63$ per 1 per cent. increase in moisture content.

Summary

Curves which represent the relationship between moisture content and respiratory rate of stored cereals are expressed mathematically by an exponential formula which approaches a linear form when the logarithm (base 10) of the rate of respiration is recorded against the arithmetical progression of moisture content. For the earlier researches on wheat the equation $y = ae^{bx} + c$ in which $x =$ moisture content and $y = CO_2$ respired, and $c =$ 0.4926 serves to express the relation between these variables between the limits of 11 and 17 per cent. of moisture. For corn or maize, the value of c in the equation becomes 0.672.

Rice and barley behave much like wheat in these particulars. Oats display less acceleration of respiration per unit increase in moisture content than do rice, barley, and wheat, while the rye samples studied deviated still more widely in this respect.

No significant correlation between market grade of barley and oats, and their rate of respiration at unit levels of moisture content appeared in the

instance of the samples included in these studies. When shriveled or small barley kernels were compared with plump or large kernels, the former registered a distinctly higher level of respiration. This relationship had previously been observed in the instance of wheat.

Flax seed respires much more vigorously than the cereals at all levels of moisture content in the range studied (11–17 per cent.).

It is suggested that this difference may result from the fact that the actual moisture content of the hydrophylic substances in the flax seed may be substantially higher than is suggested by the percentage of moisture in the entire seed.

Relative capacitance of cereal grains as measured by means of the BURTON-PITT instrument may be more highly correlated with rate of respiration than any other single chemical or physical determination heretofore employed as a basis of prediction.

The writer wishes to acknowledge the technical assistance of J. J. MARTIN and J. S. SHELLENBARGER in this study.

UNIVERSITY OF MINNESOTA
ST. PAUL, MINNESOTA

LITERATURE CITED

1. AGRONOMOFF, E. A. Temperature changes of stored grain. Scientific Institute of Cereal Research. Moscow. U.S.S.R. 1931.
2. BAILEY, C. H. The handling and storage of spring wheat. Jour. Amer. Soc. Agron. **9**: 275–281. 1917.
3. ——————. Respiration of shelled corn. Minnesota Agr. Exp. Sta. Tech. Bull. 3. 1921.
4. ——————, and GURJAR, A. M. Respiration of stored wheat. Jour. Agr. Res. **12**: 685–713. 1918.
5. ——————, and ——————. Respiration of cereal plants and grains.
   (a) II. Respiration of sprouted wheat. Jour. Biol. Chem. **44**: 5–7. 1920.
   (b) III. Respiration of rice paddy and milled rice. Jour. Biol. Chem. **44**: 9–12. 1920.
   (c) IV. Respiration of frosted wheat plants. Jour. Biol. Chem. **44**: 13–15. 1920.
   (d) V. Note on the respiration of plants infected with stem rust. Jour. Biol. Chem. **44**: 17–18. 1920.
6. BAKKE, A. L. and NOECKER, N. L. The relation of moisture to respiration and heating in stored oats. Iowa Agr. Exp. Sta. Res. Bull. 165. 1933.
7. BROWNE, C. A. The spontaneous heating and ignition of hay and other agricultural products. Science n.s. **77**: 223–229. 1933.

8. Burton, E. F. and Pitt, Arnold. A new method of conductivity measurements by means of an oscillating valve circuit. Phil. Mag. (Series 7) **5**: 939–943. 1928.

9. Coleman, D. A. and Fellows, H. C. Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. Cereal Chem. **2**: 275–287. 1925.

10. ———————, Rothgeb, B. E. and Fellows, H. C. Respiration of sorghum grains. U.S.D.A. Tech. Bull. 100. 1928.

11. Foote, H. W., and Saxton, B. The effect of freezing on certain inorganic hydrogels. Jour. Amer. Chem. Soc. **38**: 588. 1916.

12. Gilman, J. C. and Barron, D. H. Effect of molds on temperature of stored grain. Plant Physiol. **5**: 565–573. 1930.

13. Gortner. R. A. Outlines of biochemistry. p. 242. John Wiley and Sons, Inc. New York. 1929.

14. ———————. Annual review of biochemistry. **1**: 21–51. 1932.

15. Gurjar, A. M. The adaptation of Truog's method for the determination of carbon dioxide to plant respiration studies. Plant World **20**: 288–293. 1917.

16. James, L. H., Rettger, L. F., and Thom, Charles. Microbial thermogenesis II. Heat production in moist organic materials with special reference to the part played by microörganisms. Jour. Bact. **15**: 117–141. 1928.

17. Larmore, R. K., Clayton, J. S., and Wrenshall, C. L. A study of the respiration and heating of damp wheat. Canadian Jour. Res. **12**: 627–645. 1935.

18. Martin, W. McK. and Green, J. R. Determination of carbon dioxide in continuous gas streams. Ind. and Eng. Chem. Anal. Ed. **5**: 114–118. 1933.

19. Rozsa, T. A. Drinking habits of the wheat berry. The Northwestern Miller, **184**: 73–74. 1935.

20. Skovholt. Oscar and Bailey, C. H. Some new facts about molds and bread. Minnesota Agr. Exp. Sta. Bull. 296. 1933.

21. Swanson, C. O. Some factors involved in damage to wheat quality. Cereal Chem. **11**: 173–199. 1934.

22. Wilson, R. E. Humidity control by means of sulphuric acid solution, with critical compilation of vapor pressure data. Ind. and Eng. Chem. **13**: 326–331. 1921.

EFFECT OF LIGHT INTENSITY ON THE DEVELOPMENT OF THE PHOTOSYNTHETIC MECHANISM[1]

MARSTON C. SARGENT

(WITH THREE FIGURES)

Introduction

LUBIMENKO (13) was the first to observe differences in assimilatory activity between trees which ordinarily thrive only in the shade and those which thrive only in sunny places. He found that the shade species had a higher rate of photosynthesis than the sun species at low light intensities. At high intensities, however, the photosynthesis of the sun species was accelerated until it surpassed that of the shade species. He also observed that chloroplasts of the sun species, although as numerous as those of shade species, were less green and hence, he concluded, contained less chlorophyll. BOYSEN-JENSEN (2) and LUNDEGÅRDH (14), using herbaceous plants, confirmed LUBIMENKO's observations on rates of photosynthesis at high and low light intensities. JOHANNSON (12) reported in the case of a fern, and MONTFORT (15) in Cladophora that the assimilation of shade species was partially suppressed in direct sunlight. That these differences were not due in all cases to genetic factors was shown by the work of BOYSEN-JENSEN and MÜLLER (3), who found that leaves from the sunny and shady sides of a beech tree exhibited the same differences in rates of assimilation that LUBIMENKO observed. Similar phenomena, according to HARDER (11), occur in ivy leaves.

In all these cases, many external factors besides light intensity varied systematically with the environment. In order to exclude such factors, the experiments reported in this paper were carried out with the unicellular green alga *Chlorella pyrenoidosa,* which can be grown in pure culture under uniform conditions of temperature, carbon dioxide pressure, and concentration of nutrients, in light of which the wave-length distribution is maintained constant while intensity is varied at will. Using this single species of plant, grown under two selected intensities of illumination, measurements were made of (1) the maximum rate of assimilation, (2) the variation of rate of assimilation with light intensity, and (3) chlorophyll content. These measurements make it possible to revaluate the observations of earlier workers and to clarify certain characteristics of the photosynthetic mechanism which have not been emphasized in recent discussions.

[1] These experiments were conducted at the California Institute of Technology, Pasadena, and the Carnegie Coastal Laboratory, Carmel, California.

Methods

A description of the general method of cultivating Chlorella is to be found in a paper by GAFFRON (9). In the present experiments, two glass-bottomed running-water culture baths illuminated from below were used side by side in a dark room. In order to obtain "sun" and "shade" conditions, one bath was illuminated by four internally frosted incandescent lamps, burned base down with their tips 10 cm. from the bottom of the bath; the other was illuminated by a single 40-watt bulb at 17 cm. The use of bulbs of the same rating insured that the spectral composition of the light was identical, and a Weston photoelectric cell showed that the illumination on the bottom of the sun bath was seven times that of the shade bath. The total energy in the visible region falling on the bottom of the sun bath was about the same as would fall on the same area in moderately bright sunlight, but because of the difference in wave-length distribution between sunlight and the light from an incandescent filament, the two sources could not be expected to have identical effects on the complex of biological processes under discussion.

The two intensities specified were selected after a number of preliminary experiments. At intensities lower than that in the shade bath, growth was excessively slow. At intensities higher than that in the sun bath, the cultures were overheated. The intensities used were sufficiently far apart to engender marked quantitative differences in the characteristics of the algae which were of the same nature as the differences observed by earlier workers between plants in sunny or shady places.

Assimilation was measured manometrically (9) at 25° C. in Knop's solution saturated with five per cent. carbon dioxide in air. The highest illumination used was that from a closely spaced row of 100-watt bulbs at 20 cm. which is roughly comparable with bright sunlight. This intensity was reduced at need by fastening Wratten neutral filters to the bottoms of the manometer vessels, whose tops and sides were covered with brass foil. The filters were calibrated, while immersed, with a photoelectric cell, and were found to have their rated transmissions, except for the "100 per cent.," which had an actual transmission of only 93 per cent.

The concentration of chlorophyll was estimated by extracting a sample of cells with hot methyl alcohol and measuring the transmission of the extract in light of wave length 6598.953 Å from a neon tube, using a visual spectrophotometer (see EMERSON and ARNOLD (6), p. 192, for full description and determination of the standard extinction coefficient). ARNOLD and KOHN (1) found that chlorophyll from three different plants (Chlorella, Equisetum, and spinach) had the same standard extinction coefficient at this wave length. This means that the ratio of chlorophyll *a* to chlorophyll *b* is the same for these three plants within the limits of accuracy of the method of estimation, and affords no grounds for assuming that the ratio is,

in general, labile. In the present series of experiments, attempts to measure the ratio of *a* to *b* by measuring the absorption at two wave lengths failed to give reliable results.

Rates of assimilation and chlorophyll content in the present work are referred to a standard volume of cells as packed by centrifuging. When a suspension of Chlorella is centrifuged in a haematocrit for lengthening periods or at increasing velocities, the readings are found to approach a minimum value asymptotically. At 3500 r.p.m. (radius 20 cm.) and 10 minutes, small variations in velocity and time made an inappreciable difference in the volume determinations. One hundred cubic millimeters of cells have a dry weight of approximately 20 mg.

Experimental results

Early in the investigation it became apparent that while the cultures were growing, the nature of the cells of which they were composed changed progressively from day to day. A sample of cells from a freshly inoculated, thinly populated culture differed, with respect to the characteristics studied, from an old densely populated culture. Three factors in the environment can be identified as contributing to this progressive change. First, as a culture grows, the concentration of nutrient falls. Second, the average intensity of light within the culture decreases. Third, the average spectral distribution of light within the culture changes because of the selective absorption of a Chlorella suspension. Hence it is meaningless to compare a single sun culture with a single shade culture at random. Because they grow so much faster in the sun bath than in the shade bath, cultures of the same chronological age are not comparable. More nearly satisfactory is the comparison of cultures of the same population density, at least when this is low. As will be shown below, however, a suspension of sun cells transmits more light than a suspension of shade cells having the same cell concentration and this affects both the average intensity in the culture and its spectral distribution. But if cultures at all stages of the growth cycle are studied, it is found that whatever differences were present initially, persist in some degree to the end of the cycle. Determinations of the maximum rate of photosynthesis and the chlorophyll content of cells from such series of cultures were made and are here reported.

It should be emphasized that in pure cultures of *C. pyrcnoidosa* the cells do not ordinarily die. An old culture whose population has increased a thousandfold from its original size will contain no cells which, under microscopic examination, are recognizably dead or even moribund. Hence changes found by observing a sample of cell suspension can not be ascribed simply to the presence of a varying proportion of dead cells.

The fact that plants growing in the shade are greener than those growing

under high illumination can often be noted in the field, and the degree of greenness has been correlated with chlorophyll content by a number of investigators (**13, 17, 20**). The chlorophyll content of cells cultivated in the sun bath is approximately half that of shade cells. Table I gives the results

TABLE I

THE CONCENTRATION* OF CHLOROPHYLL IN CELLS FROM YOUNG SUN AND SHADE CULTURES

	Sun cultures	Shade cultures
	gm.	*gm.*
	0.026	0.071
	0.036	0.071
	0.032	0.065
	0.031	0.055
	0.033	
	0.034	
	0.037	
	0.037	
	0.035	
Mean	0.0334	0.0655
Ratio of means	$\frac{0.51}{1.00}$	

* Grams of chlorophyll per gram of cells (dry weight).

of a number of determinations expressed as grams of chlorophyll in one gram of cells (dry weight). WARBURG's data (**19**) show that under the culture conditions he specified which were similar to, but not identical with, the present ones, "Tageslichtzellen" had a chlorophyll content of 2.6 per cent. of the dry weight, while "Schattenzellen" had a content of 4.0 per cent.

TABLE II

CONCENTRATION OF CHLOROPHYLL IN CELLS FROM CULTURES OF DIFFERENT AGES

Sun cultures			Shade cultures		
Age of culture	Population density*	Chlorophyll content†	Age of culture	Population density*	Chlorophyll content†
days	*mm.*^{3}	*gm.*	*days*	*mm.*^{3}	*gm.*
3	0.11	0.033	7	0.068	0.071
4	0.37	0.034	14	0.23	0.065
6	[illegible]	0.037	23	0.65	0.078
8	1.5	0.045	30	0.95	0.073
10	1.7	0.049	37	1.3	0.063
			44	1.6	0.062
			49	1.7	0.069

* Mm.^{3} cells per cubic centimeter.
† Grams of chlorophyll per gram of cells (dry weight).

As a sun culture grows older, the chlorophyll content of a unit volume of cells increases appreciably, while in a shade culture, there is no significant change. Table II shows that while the difference between sun and shade cells is diminished in this way, it is still perfectly apparent at all stages of the growth cycle. The increase in chlorophyll content in the sun cultures can be ascribed to decrease of the average light intensity inside the cultures. Changes in the spectral distribution of the light may also play a part. From the work of EMERSON (5) and FLEISCHER (8) we should expect depletion of nutrients to act in the opposite direction, so we must conclude that this factor is either not operative, or that its effect is obscured by the light effect. Because the chlorophyll content of shade cultures is constant for all densities of population, it is possible to conjecture that at very low light intensities, chlorophyll content changes very slowly with change of intensity and approaches asymptotically a maximum value.

The maximum rate of assimilation at 25° C. that is, the rate measured at such a carbon dioxide concentration and light intensity that an increase in either of these factors (or both of them) causes no increase in assimilation is, by definition, the rate of the Blackman reaction. In cells from young sun cultures this process is about one and a half times as fast as in cells from young shade cultures. Table III shows that there is considerable

TABLE III

MAXIMUM RATE* OF PHOTOSYNTHESIS OF CELLS FROM SUN AND SHADE CULTURES

	SUN CULTURES	SHADE CULTURES
	mm.^{3} O_2	*mm.*^{3} O_2
	64	29
	56	42
	45	36
	53	39
	50	45
	65	32
	55	
Mean	55.4	37.2
Ration of means	$\frac{1.5}{1.0}$	

* Mm.^{3} oxygen per hour per mm.^{3} cells.

variation in this rate from culture to culture, but that a real difference exists between sun and shade cells. In all of the literature on Chlorella photosynthesis, no rates are reported as high as those given in table III for sun cells, and it is probable that the cells have always been grown at light intensities lower than that in the sun bath of the present experiments.

This difference between sun and shade cells is qualitatively like that

observed by previous workers (2, 13). The numerical value of the ratio depends on the exact environmental conditions specified and hence is not suitable for analytical treatment. As the difference in assimilatory rates is opposite in sense to the difference in chlorophyll content, the rate of the Blackman reaction is not a function of the chlorophyll content.

With increasing density of population the maximum rate of assimilation of both sun and shade cells decreases as shown in table IV. The rate of

TABLE IV

MAXIMUM RATE OF PHOTOSYNTHESIS OF CELLS FROM CULTURES OF DIFFERENT AGES

Sun cultures			Shade cultures		
Age of culture	Population density	Maximum photo-synthesis	Age of culture	Population density	Maximum photo-synthesis
days	*mm.*^{3}	*mm.*^{3} O_2	*days*	*mm.*^{3}	*mm.*^{3} O_2
4	0.27	56	7	0.068	34
5	0.90	50	14	0.23	31
6	1.3	27	23	0.65	25
8	1.9	18	30	0.95	18
11	2.5	13	37	1.3	13
13	3.0	10	44	1.6	12
17	3.3	9.9	49	1.7	11

decrease is most marked in sun cells so that old cultures are indistinguishable from old shade cultures. Unless a sun culture is examined while it is still thinly populated (that is, while the average internal illumination is high, the color of the light essentially white, or the supply of nutrients undiminished) the difference between it and a shade culture might escape attention completely. Only when surrounded by these conditions does an actively dividing cell population suddenly build up its collective capacity for carrying on the Blackman reaction.

It might be thought that in a rapidly dividing population, the cells would be smaller than in a more stable population and that consequently, in a unit volume of cell material, the total surface of all the cells would be large. The rate of the Blackman reaction might then turn out to be proportional to this total surface or to the total area of some active surface (such as that of the chloroplasts) inside the cells. The cells of *Chlorella pyrenoidosa* are too small (diameter 3 to 5 μ) to measure accurately but it is easy to count in a haemacytometer the number of cells in a measured volume of culture medium and thence to calculate the number in a cubic millimeter packed by centrifuging. Table V shows such counts for sun and shade cultures at all stages of the growth cycle. Work by YIN in this laboratory has shown in the case of *Chlorella vulgaris* that the number of cells in a unit packed

TABLE V

SIZE OF CHLORELLA CELLS IN CULTURES OF DIFFERENT AGES

Sun cultures		Shade cultures	
Population density	Number of cells in 1 mm.³ packed volume	Population density	Number of cells in 1 mm.³ packed volume
mm.³ cells per ml.		*mm.³ cells per ml.*	
0.19	21×10^6	0.068	23×10^6
0.12	24	0.23	27
0.99	19	0.65	22
0.98	18	0.95	21
0.95	18	1.3	17
1.43	15	1.6	18
1.23	15	1.7	17
Mean	18.6		20.7

volume is determined only by the size of the cells, and not by differences in the closeness of packing. Hence it can be shown that the total area of the surfaces of all the cells in one cubic millimeter is proportional to the cube root of the number of cells. From table V it is apparent that sun cells are so nearly the same size as shade cells that the difference between the cube roots is negligible. Cells from old cultures are, on the average, larger than those from young ones, but the difference is by no means large enough to account for the difference in the rate of the Blackman reaction on the basis of a difference in surface area.

Not only at saturating light intensities, but also at all lower intensities, the rate of assimilation of sun cells is higher than that of shade cells. Figure 1 shows the relation of photosynthesis to light intensity for samples of each variety of cells. The data for these curves and others appear in table VI. It is clear that while the ratio between the rates of sun and shade plants decreases with decreasing intensity, it never drops below 1.0 even in the range where photosynthesis is proportional to light intensity. As previous investigators, without exception, have reported that at low light intensities, shade plants assimilate faster than sun plants, this result is unexpected.

When an attempt is made to compare rates of photosynthesis of cell suspensions at sub-saturating intensities, it is necessary to take into account the decrease of intensity suffered by light traversing the suspension. The laminae of the suspension nearest the source of illumination shade the laminae behind them. This shading would be represented in figure 1 by moving all the points on a curve to the left by an amount proportional to their distances from the y-axis. If the corrections were very different for

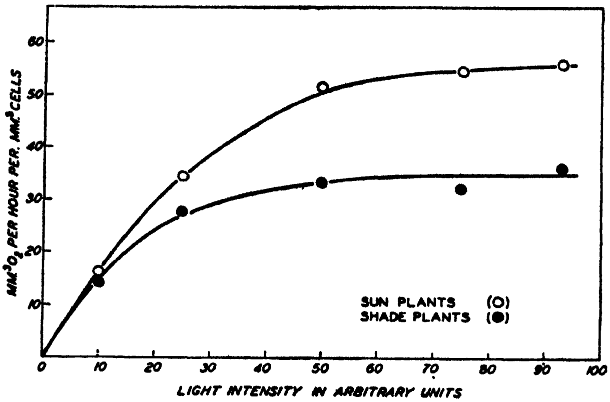


FIG. 1. Rate of photosynthesis of sun and shade plants at different light intensities. The approximate value of the light intensity marked 100 is discussed in the text.

PROTOCOLS FOR FIGURE 1

SUN PLANTS, AUGUST 13, 1936

CULTURE FOUR DAYS OLD. INOCULATED WITH 0.2 MM.³ CELLS. POPULATION DENSITY, 0.27 MM.³ CELLS IN 1 ML. 1.9 MM.³ CELLS IN EACH VESSEL. TEMPERATURE, 25°

Filter	10%	25%	50%	75%	93%
Reading (60′ light) mm. Brodie	35.6	82.0	73.6	73.6	76.8
Vessel constant	0.750	0.744	1.27	1.35	1.33
O_2 evolved (60′ light)	26.7	61.0	93.6	99.5	102.1
O_2 consumed (60′ dark)	4.3	4.3	4.3	4.3	4.3
Photosynthesis per vessel	31.0	65.3	97.9	103.8	106.4
$mm.^3$ cells per vessel	1.9	1.9	1.9	1.9	1.9
$mm.^3$ O_2 per hour per $mm.^3$ cells	16.3	34.4	51.5	54.6	56.0

SHADE PLANTS, AUGUST 27, 1937

CULTURE TWELVE DAYS OLD. INOCULATED WITH 0.6 MM.³ CELLS. POPULATION DENSITY, 0.32 MM.³ CELLS IN 1 ML. 1.1 MM.³ CELLS IN EACH VESSEL. TEMPERATURE, 25°

Filter	10%	25%	50%	75%	93%
Reading (60′ light) mm. Brodie	11.3	21.9	26.3	45.8	51.9
Vessel constant	1.27	1.35	1.33	0.750	0.744
O_2 evolved (60′ light)	14.4	29.6	35.0	34.4	38.6
O_2 consumed (60′ dark)	1.3	1.3	1.3	1.3	1.3
Photosynthesis per vessel	15.7	30.9	36.3	35.7	39.9
$mm.^3$ cells per vessel	1.1	1.1	1.1	1.1	1.1
$mm.^3$ O_2 per hour per $mm.^3$ cells	14.3	28.1	33.0	32.4	36.3

the two curves, the latter might cross. The principal agents of this shading (absorption, and scattering) cannot be eliminated by any practical method or adjusted to equivalence for different suspensions. They can, however, be minimized. The data in table VI were obtained with suspensions contain-

TABLE VI

THE RATE OF PHOTOSYNTHESIS OF CELLS FROM YOUNG SUN AND SHADE CULTURES AT VARYING LIGHT INTENSITIES. PHOTOSYNTHESIS AND RESPIRATION IN VOLUMES OF OXYGEN PRODUCED BY UNIT VOLUME OF CELLS IN AN HOUR. LIGHT INTENSITIES IN UNITS PROPORTIONAL TO ABSOLUTE INCIDENT INTENSITIES.

	LIGHT INTENSITY					
	10	25	50	75	93	DARK
Sun cultures	16	34	52	55	56	-2.3
	23	44	55	58	61	-0.6
	17	37	55	61	65	-1.6*
Shade cultures	14	28	33	32	36	-1.2*
	17	30	33	38	39	-1.5*
	14	25	26	24	26	-1.8

* The respiration and respiratory quotient were determined in a separate experiment.

ing less than 2 mm.^{3} of cells in the volume of fluid used in each vessel. Each vessel had a bottom area of about 5 cm.^{2} The diameters of Chlorella cells range from 3 to 5 μ. A simple calculation shows that if all the cells settle uniformly they would form a layer only one cell deep, which would not entirely cover the bottom of the vessel. In a suspension of such meager density the average illumination within the vessel should be nearly identical with the incident illumination. Hence the correction to be applied to the points in figure 1 would be small and would not affect the relative positions of the two curves.

Another possible source of error is the correction for respiration. The rate of respiration, however, in the present experiments was, in every case, small compared with the rate of photosynthesis at even the lowest light intensity used. In those cases marked with an asterisk in table VI the rates of respiration and the respiratory quotient were separately determined with great care. They, as well as all other respiration rates measured in this investigation, were of the same order of magnitude and of an order lower than the rate of photosynthesis. The modifications necessary to change the relative positions of any parts of the curves in figure 1 are larger than any possible errors that could have occurred.

There seems to be no question that sun cells of Chlorella assimilate faster than shade cells even at low light intensities. Before concluding that there is a fundamental difference between Chlorella and the sun and shade plants

investigated by earlier workers, we must examine their experiments in detail. The statements of LUBIMENKO, BOYSEN-JENSEN, and LUNDEGÅRDH are based on readings obtained in the light, not corrected for respiration. They found, however, in every case, that sun plants have a higher rate of respiration than shade plants. The magnitude of the difference is such that, if the readings at low light intensities are corrected by these amounts, the sun plants at every light intensity have a higher true rate of photosynthesis than the shade plants. The conclusion of LUBIMENKO, (**13**), and others, that shade plants are "adapted" to existence in shady places is not affected by this fact, but in terms of the present argument their sun plants assimilated faster than their shade plants at all light intensities.

Shade cells of Chlorella show no inhibition of photosynthesis by light of high intensity. In table VI appear the figures for cells which were saturated by light intensity 25, and showed undiminished photosynthesis under four times as much illumination. Other cases more marked than this were observed. While in very intense light, the shade variety of Chlorella might suffer an inhibition of photosynthesis, no inhibition is apparent at intensities like that of direct sunlight, or at intensities ten to twenty times that in which the cells have grown. There is no sharply optimal intensity for the assimilation of the shade cells as found by MONTFORT and NEYD: L (**16**) for shade varieties of ferns.

The rate of photosynthesis of sun and shade varieties of Chlorella at different concentrations of carbon dioxide was measured in mixtures of tenth molar potassium carbonate and potassium bicarbonate as described by EMERSON and ARNOLD (**6**). The partial pressures of carbon dioxide in equilibrium with these solutions have been calculated from figures given by WALKER, BRAY and JOHNSTON (**18**). The interpretation of results obtained

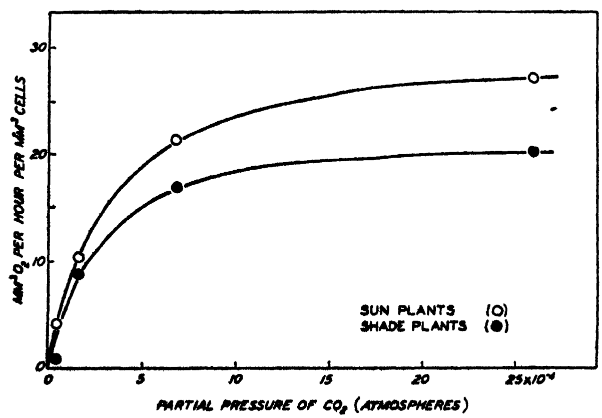


FIG. 2. Rate of photosynthesis of sun and shade plants at different carbon dioxide tensions.

by the use of carbonate-bicarbonate mixtures is at present open to doubt (7). In the absence of a fully accepted method of measuring photosynthesis at low carbon dioxide concentrations, however, data obtained by the present method are at least comparable with much published data obtained by the same method.

The curves relating photosynthesis of sun and shade plants to carbon dioxide concentration appear to be of the same shape (fig. 2). They could be made to coincide by multiplying all the points of either curve by a factor. This factor (1.3 or its reciprocal) is the same as that which relates the rates of the Blackman reaction for the two varieties of plants. Whatever process (or complex of processes) gives the rates measured here, the effect of the sun or shade environment on it is the same as that on the velocity of the Blackman reaction. The rate of carbon dioxide utilization at all partial pressures is independent of the chlorophyll concentration.

The effect of cyanide on the assimilation of sun and shade plants is shown in table VII. The velocity of the Blackman reaction in the sun

TABLE VII

THE EFFECT OF HCN ON PHOTOSYNTHESIS OF CELLS FROM YOUNG SUN AND SHADE CULTURES

	Concentration of HCN	Rate of photosynthesis*	Inhibition
	molar	$mm.^3$ O_2	%
Sun	0	42	0.0
	1×10^{5}	39	5.3
	5 "	30	29
	10 "	19	54
	50 "	6.7	83
Shade	0	21	0.0
	1×10^{-5}	20	6.7
	5 "	16	27
	10 "	10	51
	50 "	4.3	80

* $Mm.^3$ oxygen per hour per $mm.^3$ cells.

plants in this case is twice that in the shade plants, as shown by the values for photosynthesis without cyanide, but the percentage inhibition by any given concentration of cyanide is the same in both varieties of cells.

The photosynthetic quotient, that is, the ratio of carbon dioxide consumption to oxygen production in the light, is the same in sun and shade plants. Within the limits of experimental error it is equal to -1.00. The respiratory quotient, the ratio of carbon dioxide to oxygen in the dark, is numerically larger. Table VIII shows that this is the case especially in shade plants. As the calculation of rates of photosynthesis is generally

TABLE VIII

THE RESPIRATION OF CELLS FROM YOUNG SUN AND SHADE CULTURES

	RESPIRATORY QUOTIENT	RATE OF RESPIRATION*
Sun	−1.03	3.4
	−1.17	1.8
	−1.02	1.6
Shade	−1.51	0.9
	−1.30	1.2
	−1.85	1.5

* $Mm.^3$ O_2 consumed by 1 $mm.^3$ cells in one hour.

based on the assumption that the respiratory quotient is −1.00, this fact would introduce an error, but the rate of respiration is so small compared with that of photosynthesis that the error is negligible. In the only case in which it might be important, the rate of oxygen consumption in the dark was determined directly (table VI). As shown in table VIII, the absolute rate of respiration of sun plants is somewhat higher than that of shade plants.

The actual rates of growth of sun and shade cultures are of some interest. Curves representing these rates are shown in figure 3. The shade

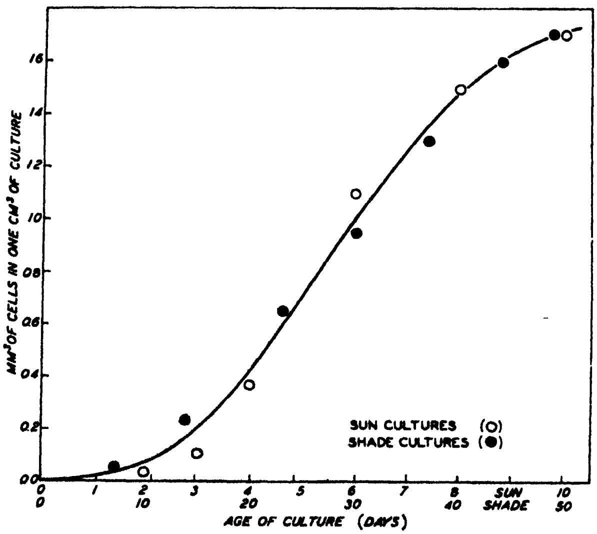


FIG. 3. Growth of Chlorella cultures under sun and shade conditions. The time coordinate for shade cultures is five times as long as that for sun cultures.

cultures in this experiment were grown at a higher light intensity than those reported elsewhere in this paper, namely at 11 cm. from a 40-watt bulb, so that the ratio of the illumination in the sun bath to that in the

shade bath was somewhat higher than 4 to 1. While the ordinates for both curves are the same in figure 3, the abscissae of the shade curve are five times as long as those of the sun curve. By the inclusion of this factor, the curves are superposed except near the origin, which means that the sun cultures grew five times as fast as the shade cultures. This ratio is very near the ratio between the illuminations in the two baths. Unless it is assumed gratuitously that one kind of cells contains more water than the other, this is a measure of the relative rates of photosynthesis. Hence the cells in the cultures assimilate at rates which fall on the rate-intensity curves of figure 1 in the range where assimilation is proportional to light intensity, and the light intensity at which they exhibit maximum photosynthesis is considerably higher than that in which they have been cultivated. The effect of both sun and shade environments is to produce cells whose maximum capacity for assimilation is never realized in their environments.

Discussion

These experiments show that differences in illumination are sufficient to cause the differences between sun and shade plants observed by earlier workers as noted in the introduction above. When other factors are maintained uniform, plants grown in bright light have a higher rate of photosynthesis and lower chlorophyll content than plants grown in the shade.

Shade plants may have such a low rate of respiration that their excess of photosynthesis over respiration at low light intensities is higher than that of sun plants. In the present experiments shade cultures of Chlorella do not show this "adaptation" to a shade existence but there is a tendency in that direction.

The light optimum for shade plants observed by JOHANNSON (**12**) and by MONTFORT (**15**), does not appear in the present experiments and in those of BOYSEN-JENSEN, LUNDEGÅRDH, and others (**2**, **14**). Moreover, optima have been reported for plants which are distinctly sun varieties, namely marine diatoms; thus if optima can be considered as occurring at all, they are not characteristic of shade plants alone. Recently reported experiments by F. GESSNER (**10**) give sound reasons for believing that light optima are artifacts arising from experiments in sealed containers in which insufficient care is taken to avoid stagnation, carbon dioxide deficiency, and consequent damage to the photosynthetic mechanism.

The present experiments give no support to the idea that the lower chlorophyll content of sun plants is to be attributed to the existence of a light sensitive process in which chlorophyll is decomposed. From the rates of growth of sun and shade cultures, and the chlorophyll content of the cells, it follows that during any given period, a unit volume of sun cells produced about two and a half times as much chlorophyll as the same vol-

ume of shade cells. Not all of the implications of this situation are simple but at least it affords no reason for believing that there occurred a continuous decomposition of chlorophyll under the influence of light, which proceeded more rapidly in the sun cultures than in the shade cultures. These experiments however, do not exclude the possibility that such a process occurs.

The present experiments have some bearing on ecological problems. The blooming of marine diatoms and fresh-water algae occurs when the supply of mineral nutrients is large and in a season of intense or prolonged insolation. Under such conditions photosynthesis and, consequently, growth and cell division are rapid. In addition, it appears likely that under these favorable conditions there will be produced plants especially capable of a high rate of photosynthesis. That is, the population of shade plants (or low-nutrient plants) will produce a population of sun plants whose individual capacity for photosynthesis is considerably higher than that of the original population. When the period of favorable conditions comes to an end through either depletion of nutrients or decrease of insolation, the plants will change into the low-nutrient or shade form and their lowered capacity for photosynthesis will compound the effect of the unfavorable conditions. Such a compounding of effects may go far to explain the sudden burst and sudden cessation of growth characteristic of blooms.

That some chlorotic plants reach maturity while other individuals of the same species die early (although by the action of genetic factors, they contain much more chlorophyll (4)) may, in some cases, be attributed to processes discussed in this paper. Some individuals genetically poor in chlorophyll may have highly efficient photosynthesis and an unimpaired capacity for the Blackman reaction. At both high and low light intensities they could assimilate as rapidly as individuals with much more chlorophyll. The rate of the Blackman reaction is easily determined and has been measured successfully in a great variety of plants. A study of the inheritance of this factor might yield important information.

Summary

Chlorella pyrenoidosa cultivated under intense illumination has a low chlorophyll content, and a high capacity for photosynthesis. Cultivated under moderately low illumination it has a high chlorophyll content, and a low capacity for the Blackman reaction. The proportional effects of carbon dioxide concentration and cyanide are the same in both varieties of plants. The photosynthetic quotient in both sun and shade plants is equal to -1.00. The respiratory quotient is absolutely larger especially in shade cells. The average cell size of sun and shade plants is very nearly the same. The rate of growth of the cultures is approximately

proportional to the intensity of illumination. As the population density of the culture increases, the cells undergo internal changes. The average cell size increases; the chlorophyll content (in sun cells) increases; the capacity for the Blackman reaction decreases.

The author expresses his indebtedness to MR. ROBERT EMERSON and MR. LOWELL F. GREEN for numerous suggestions and constant assistance in carrying out these experiments.

SCRIPPS INSTITUTION OF OCEANOGRAPHY
LA JOLLA, CALIFORNIA

LITERATURE CITED

1. ARNOLD, WILLIAM, and KOHN, HENRY I. The chlorophyll unit in photosynthesis. Jour. Gen. Physiol. **18**: 109–112. 1934.
2. BOYSEN JENSEN, P. Studies on the production of matter in light- and shadow-plants. Bot. Tidsskr. (Copenhagen) **36**: 219–259. 1918.
3. ——————, and MÜLLER, D. Über die Kohlensäureassimilation bei Marchantia und Peltigera. Jahrb. wiss. Bot. **70**: 503–511. 1929.
4. DEMEREC, M. Behavior of chlorophyll in inheritance. Cold Spring Harbor Symp. Quant. Biol. **3**: 80–86. 1935.
5. EMERSON, R. The relation between maximum rate of photosynthesis and concentration of chlorophyll. Jour. Gen. Physiol. **12**: 609–622. 1929.
6. ——————, and ARNOLD, WILLIAM. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.
7. ——————, and GREEN, LOWELL. Effect of hydrogen-ion concentration on Chlorella photosynthesis. Plant Physiol. **13**: 157–168. 1938.
8. FLEISCHER, WALTER E. The relation between chlorophyll content and rate of photosynthesis. Jour. Gen Physiol. **18**: 573–597. 1935.
9. GAFFRON, H. Methoden zur Untersuchung der Kohlensäureassimilation. Abderhalden's Handbuch der biologischen Arbeitsmethoden **11**: 101–160. 1929.
10. GESSNER, F. Die Beziehung zwischen Lichtintensitat und Assimilation bei submersen Wasserpflanzen. Jahrb. wiss. Bot. **86**: 491–526. 1938.
11. HARDER, R. Bemerkungen über die Variationsbreite des Kompensationspunktes beim Gaswechsel der Pflanzen. Ber. d. bot. Ges. **41**: 194–198. 1923.
12. JOHANNSON, N. Zur Kenntnis der Kohlensäureassimilation einiger Farne. Svensk Bot. Tidskr. **17**: 215–223. 1923.
13. LUBIMENKO, M. W. Sur la sensibilité de l'appareil chlorophyllien des

plantes ombrophiles et ombrophobes. Rev. Gén. Bot. **17**: 381–415. 1905.

14. LUNDEGÅRDH, H. Ecological studies in the assimilation of certain forest-plants and shore-plants. Svensk Bot. Tidskr. **15**: 46–95. 1921.
15. MONTFORT, CAMILL. Die funktionelle Einstellung verschieden gefärbten Meeresalgen auf die Lichtintensität. Jahrb. wiss. Bot. **71**: 106–148. 1929.
16. ———, and NEYDEL, KARL. Zur Beurteilung der "Inaktivierung" und des "Zeitfaktors" bei der Assimilation Stomatafreier Schatten-Farne. Jahrb. wiss. Bot. **68**: 801–843. 1928.
17. STÅLFELT, M. G. Zur Kenntnis der Kohlehydratproduktion von Sonnen- und Schattenblättern. Medd. från Statens Skogsforsoksanst. **18**: 221–280. 1921.
18. WALKER, A. C., BRAY, U. B., and JOHNSTON, J. Equilibrium in solutions of alkali carbonates. Jour. Amer. Chem. Soc. **49**: 1235–1256. 1927.
19. WARBURG, O. Über die Katalytischen Wirkungen der Lebendigen Substanz. Berlin. 1928.
20. WILLSTÄTTER, R., and STOLL, A. Untersuchungen über die Assimilation der Kohlensaure. Berlin. 1918.

AUXIN IN MARINE ALGAE

J. VAN OVERBEEK

(WITH ONE FIGURE)

Introduction

Auxin has been studied almost exclusively in the higher land plants. Therefore, it seemed of interest to obtain some information regarding auxin in very different groups such as the lower water plants. For this purpose Macrocystis was selected. *Macrocystis pyrifera* (L.) Ag. is a large brown alga growing on rocks, usually in 10 to 30 meters of water. A description can be found in SETCHELL and GARDNER (**11**), or OLTMANNS (**10**). The stipe ("stem") becomes 30 to 50 meters long, bearing at its tip a terminal, bladderless, falcate blade and lower down the young blades and along the greater part of its length at regular intervals the mature, lateral blades, each with a pyriform bladder at its base. The mature blades are from 3 to 5 dm. long and 5 to 9 cm. wide. The upper 2 to 10 meters of the thallus usually floats on the surface of the water. The alga is perennial, which offers the advantage that material can be collected throughout the year. Since it was not considered practical to investigate the entire plant, only a young apical part about 50 cm. in length was used. Such a part consists of a terminal blade and a stipe to which are attached on the average 15 blades and bladders of an increasing size toward the base. Before the physiological rôle of auxin of a plant can be determined it is desirable to: (a) demonstrate the presence of auxin; (b), to determine its distribution throughout the plant; and (c) to discover the type of the auxin involved. These are the three points which will be discussed in this paper.

VAN DER WEIJ (**12**) in 1933, for the first time demonstrated the presence of auxin in marine algae. In the cell sap of young *Valonia macrophysa* plants he found between 0.00012 and 0.00021 mg. of auxin per liter, and in the cell walls of the same material about twenty times that amount. DU BUY and OLSEN (**3**) extracted auxin from Fucus. During the summer of 1938 I determined the auxin content of the green alga Bryopsis which had been collected and extracted (**14**, **15**) by MR. M. L. DARSIE at Pacific Grove, California. About 80 gamma equivalents of indole acetic acid per kg. fresh weight were found. This is an auxin concentration of the same order as occurs in pea seedlings, but considerably smaller than found in Avena and corn plants (**13**, **14**, **15**). Another water plant in which such relatively large auxin concentrations were found is Elodea, in which I found auxin concentrations as high as 50 gamma equivalents of indole acetic acid per kg. fresh weight. In Macrocystis, on the other hand, auxin concentrations

of only about one gamma per liter were found, which is close to VAN DER WEIJ's values for Valonia.

Experimental methods and results

Young undamaged plants free from overgrowths, as far as could be determined macroscopically, were collected in a pail of sea water. The plants were collected by boat at the kelp beds near the breakwaters of Newport Bay, or off the coast. Material that had become detached or was washed ashore was never used. Within one half hour after collection, the material was thoroughly washed with fresh water in order to remove microorganisms that might be attached, and then placed in highly purified ethyl ether. It was left in the ether for 2 to 3 days, and after that time the assay for auxin was made in a previously described manner (**14**, **15**). However, one further simplification was introduced (suggested by DR. W. S. STEWART). Instead of evaporating the residue to complete dryness and adding agar to it, the extract was evaporated down to about 2 ml. which were then dropped immediately into the hot agar. In this way the auxin was instantaneously mixed with the agar and the 2-hour period of standing which was previously necessary to insure this mixing was eliminated. Each test included a control curve with known amounts of indole acetic acid which made it possible to bring the threshold value into calculation (**16**).

In order to check whether or not all of the auxin was removed, the extraction method described above was compared with the Soxhlet method, which is one of the most exhaustive methods known. Table I shows that practically the same amount was obtained by both methods. Hence, one can be reasonably sure that the data presented here truly represent the auxin content of the material.

TABLE I

AMOUNTS OF AUXIN* OBTAINED FROM MACROCYSTIS BLADES BY MEANS OF EXTRACTION IN ETHER FOR 2 DAYS AND BY SOXHLET EXTRACTION DURING THE SAME TIME (81101)

PART OF PLANT	SOXHLET	STANDING
Terminal blade	0.61	0.73
Young lateral blades	1.13	1.00

* Gamma equivalents of indole acetic acid per kilogram fresh weight.

Distribution

As was stated in the introduction, only the apical 50 cm. of the thallus was investigated. In table II a typical distribution experiment is represented which shows all of the individual factors necessary for the determination of the auxin concentration in the plant. In the first column is indicated the part of the thallus and the number used for extraction. The column marked W indicates the total fresh weight in grams of the parts

TABLE II

A COMPLETE ANALYSIS OF THE AUXIN DISTRIBUTION IN THE APICAL PART OF A MACROCYSTIS THALLUS. EXPLANATION OF SYMBOLS IN TEXT (81026)

	W	V_a	C	$(C \times I_{1^\circ} + O)$	$\frac{\text{GAMMA}}{\text{KG.}}$	$\frac{\text{GAMMA}}{\text{PART}}$
	gm.	*ml.*	0°			
10 terminal blades	17.5	0.5	6.5	19.0	0.54	87×10^{-5}
			5.3	16.0	0.46	
10 stipes	42.0	0.3	7.2	20.0	0.14	59×10^{-5}
100 bladders	59.0	0.5	6.8	19.5	0.16	9×10^{-5}
			7.0			
15 lateral blades	62.0	0.5	7.0	20.0	0.16	78×10^{-5}
			10.5	27.5	0.22	

Controls

26.5 gamma indole acetic acid per liter gave $C = -10.0°$
13.2 gamma indole acetic acid per liter gave $C = -\ 4.1°$
0.0 gamma indole acetic acid per liter gave $C = +\ 1.8°$

under investigation. The column V_a indicates the volume of agar in ml. in which the extract was taken up, C gives the degree of curvature in the Avena test. The factor $(C \times I_{1^\circ} + 0)$ translates degrees of curvature into concentration of indole acetic acid. Its value can be directly determined from the concentration curve with known amounts of indole acetic acid. I_{1° is the indole acetic acid concentration required to give an increase of curvature in the Avena test of 1°. 0 is the threshold value in gammas indole acetic acid per liter. The auxin concentration in gamma equivalents of indole acetic acid per liter (kg. fresh weight) is calculated from:

$$\frac{(C \times I_{1^\circ} + 0) \times V_a}{W}$$

which is given in the sixth column. The last column finally gives the auxin content of an average terminal blade, stipe, bladder, or young lateral blade.

As table II indicates, the auxin concentration is highest in the terminal blade and lowest in the stipe. This relation was generally found, but the terminal blade does not always have a higher auxin concentration than the upper lateral ones, as follows from table I. In table III a summary of all available data on the auxin distribution is given. There appears to be a definite decrease in auxin concentration in the basal direction although this auxin gradient is not as pronounced as in seedlings of higher plants (14, 15). The low auxin concentration in the rapidly growing stipe may be compared with a similar situation in young corn seedlings (15). Here it was found that the upper part of the first internode, which is the fastest

TABLE III

DISTRIBUTION OF AUXIN* IN THE UPPER PART OF THE THALLUS OF MACROCYSTIS

TERMINAL BLADE	YOUNG BLADES	OLDER BLADES	BLADDERS	STIPE	EXPERIMENT NUMBER	DATE
1.75	1.00	0.53	......	......	80926	(Sept. 26, 1938)
0.84	0.19	......	......	0.21	81019	(Oct. 19, 1938)
0.51	....	....	......	......		
0.54	0.16	....	.16	0.14	81026	(Oct. 26, 1938)
0.46	0.22	....	......	......		
0.61	1.13	....	......	....	81101	(Nov. 1, 1938)
0.73	1.00	....	......	......		

* Gamma equivalents of indole acetic acid per kilogram of fresh weight.

growing region of the entire corn seedling, has the lowest auxin content. It may be that in these cases the rapidly growing tissue consumes large amounts of auxin as discussed later.

TYPE OF AUXIN

In order to determine the nature of the auxin present in Macrocystis three types of investigation are open.

(a). The most satisfactory is to obtain it in crystalline form and determine its structure in a manner similar to that followed by KÖGL and HAAGEN-SMIT for auxin-a,-b, and indole acetic acid. A glance at tables I, II, and III shows that only about one-half part of auxin per billion (10^9) parts of Macrocystis is present. Hence it would require tons of material to isolate a fraction of a gram of auxin, which makes direct determinations impracticable in this case.

(b) KÖGL, HAAGEN-SMIT, and ERXLEBEN (7) have described a convenient differential acid-alkali destruction test which makes it possible to distinguish between indole acetic acid which is destroyed by acid but stable in alkali, auxin-a which is stable in acid but destroyed by alkali, and auxin-b which is unstable both in acid and alkali. When this method was tried on extracts of Macrocystis blades, invariably the auxin present in it was destroyed by acid, but not by alkali, indicating that indole acetic acid was present. Table IV gives the results of a few experiments of this type. It is noteworthy that in several instances refluxing with sodium hydroxide increased the auxin activity of the extract over that which had been boiled with distilled water. It may be that by boiling with dilute NaOH active auxin is liberated from a precursor, but it is also possible that some inhibitory substance is inactivated. Since HAAGEN-SMIT (private communication) does not consider the differential destruction test reliable for impure ex-

TABLE IV

AUXIN CONTENT* OF MACROCYSTIS EXTRACTS

REFLUXING WITH 5 PER CENT. HCL	REFLUXING WITH 1 N NAOH	REFLUXING WITH DISTILLED WATER	CONTROL NOT REFLUXED	TIME	EXPERIMENT NUMBER
deg.	*deg.*	*deg.*	*deg.*	*min.*	
+ 0.1	11.3	5.5		45	81025
+ 1.5	10.0	6.3			
2.3	14.1	12.3	12.8	30	81019
2.9	13.5	10.7	11.6		

* Degrees of curvature in the Avena test.

tracts, the third method which is available for obtaining information about the nature of the auxin was also tried.

(c) This method is based upon the determination of the diffusion coefficient and was first successfully applied by WENT (**17**), who four years before the auxin-a was chemically isolated, had determined its molecular weight. The experiment was carried out as follows: the auxin present in an extract of Macrocystis was taken up in a small agar plate ($10.8 \times 8.1 \times 0.8$ mm.). This plate was then carefully placed on top of a stack of three similar plates containing no auxin. After 40 minutes the 4 plates were separated and the auxin content in each of them determined by the Avena test method. From the relative distribution of the *actual amount* of auxin (rather than Avena curvature) in each of the four plates the diffusion coefficient can be found from diffusion tables after SHEFFER and KAWALKI (**1**). Once the diffusion coefficient (D) is known, the molecular weight can be calculated from $\sqrt{M} = \frac{7 \times 1.07}{D_{22^\circ}}$. Since it is extremely important to know the relative distribution of the actual auxin content in the four agar plates, one is not justified in directly using the degrees of curvature obtained in the Avena test for the determination of the relative auxin distribution in the tables, unless one is sure that there is a direct proportionality between the Avena curvature and the actual auxin concentration in the agar plates. Such a direct relationship does not exist in a great many cases. The curve did not start at the origin, but somewhere on the concentration axis, indicating that there was a threshold value. In order to avoid this error a diffusion test was run with indole acetic acid ($M = 175$) parallel to the diffusion test with the unknown auxin. In this control run the expected relative distribution of the indole acetic acid in the 4 agar plates may be found from the tables. The Avena curvature for each of these 4 plates is found by means of the Avena test. When these Avena curvatures are

plotted against the concentration of auxin obtained from the tables an accurate concentration curve for the conditions of the experiment is obtained. This curve subsequently was used to translate the Avena curvature of the diffusion test with the unknown auxin into relative amounts of auxin. When this was done diffusion coefficients and molecular weights for the auxin in Macrocystis were found which were close to those of indole acetic acid (table V). The difference between the molecular weight found for

TABLE V

DIFFUSION AND MOLECULAR WEIGHTS FOR AUXIN IN MACROCYSTIS. EXPLANATION IN TEXT

	$D_{22°}$	MOLECULAR WEIGHT	EXPERIMENT NUMBER
Auxin-a	0.414	328	......
Auxin-b	0.426	310	......
Indole acetic acid	0.567	175	......
Auxin from Macrocystis	0.612	149	81117
	0.599	156	81207

Macrocystis-auxin and that of indole acetic acid is probably not significant. It is clearly shown, however, that the auxin of Macrocystis cannot be auxin-a or -b.

In determining the molecular weight by means of the diffusion test it was found inadvisable to work with a donor plate which contained an excessive amount of impurities. Such a block gives too low values in the Avena test. It was found that when such an impure block was placed for one hour on top of a block of plain agar, the latter gave a higher curvative than the former. Auxin had diffused into this plate of agar, but the majority of impurities remained behind in the original block. In the experiments mentioned in table V, use was made of this simple method of purification.

Discussion

It is generally assumed that higher plants contain auxin-a and -b and that lower ones contain indole acetic acid. This generalization is based upon the actual isolation of auxin-a and -b from corn oil and malt (**6**), and by a number of molecular weight determinations for higher plants (**4**, **7**) all of which showed that auxin of a molecular weight like that of auxin-a or -b is present in these plants. The lower plants so far investigated have been fungi and bacteria. Indole acetic acid was actually isolated from yeast and Rhizopus (**2**, **5**, **7**), and more indirect evidence for the presence of indole acetic acid was obtained in Aspergillus and *Bacterium coli* (**9**, **10**). The lower plants so far investigated were saprophytes in contrast to the

autotrophic higher plants. It is interesting to see that in the case of Macrocystis, an autotrophic lower plant, indole acetic acid or a substance closely related to it is present.

Although auxin has now been demonstrated in at least 3 marine algae, its mere presence does not make it a growth hormone for these organisms. Further researches have been planned in order to elucidate this particular point. There is evidence, however, that auxin is a growth hormone for Macrocystis: (1) the higher auxin concentration in the blades of the apical growing part of the thallus than in the older mature part. (2) The similarity in the relation between growth and auxin distribution in corn seedlings and in the stipe of Macrocystis. In an earlier paper (**15**) it was shown that in young corn seedlings the region of maximal rate of elongation is located in the upper part of the first internode. It was also shown that out of this particular region the smallest amount of auxin of the entire plant could be extracted. Strange though this may seem at first sight the following consideration will show that it is to be expected. If one assumes that auxin is used up during the process of elongation one can expect that the faster a particular region elongates the more auxin it uses. In the upper region of the corn coleoptile the growth rate is relatively small and is not limited by auxin but by other factors. In the lower part of the coleoptile and the upper part of the first internode elongation is limited by auxin. This has been shown by decapitation experiments (**15**). In this upper region of the first internode the other factors necessary for elongation are present in excess, and auxin will cause it to elongate as soon as it arrives at the proper spot. It is thereby rendered inactive. When active auxin is extracted it will be auxin which was present in the plant under the following conditions: (a) on its way to those regions where it will cause elongation; (b) on its way to more basal regions; and (c) as bound auxin. For a discussion of bound and free-moving auxin see (**18**) and a paper by the writer on "Auxin in roots."[1]

It will be clear from the above consideration that in the upper part of the coleoptile, where the auxin is produced and relatively little is used for elongation, relatively large amounts are found upon extraction. On the other hand, the more basal parts of the corn seedling (the upper part of the first internode) will have a relatively low auxin concentration because: (1) they receive only auxin which has been left over by the more apical regions; and (2) since the other growth factors are present in excess, practically all the auxin will be used for elongation. If a similar relation between elongation and auxin content exists in the stipe of Macrocystis, the relatively low auxin content there may be regarded as an indication that its elongation is auxin controlled.

[1] Bot. Gaz. **101**: 450–456. 1939.

Summary

In the brown alga Macrocystis auxin is present in a concentration of approximately 0.5 gamma equivalents of indole acetic acid per kilogram fresh weight. In the green alga Bryopsis up to 80 gammas per kilogram, and in Elodea 50 gammas per kilogram fresh weight were found. These are auxin concentrations of the same order of magnitude as are present in higher plants such as corn (on the average 0.5 gamma equivalents of indole acetic acid per kg.) and pea seedlings (on the average 50 gammas per keg.). The auxin distribution in the upper 50 cm. of the thallus was investigated. The young blades had the highest auxin concentration and the stipe (stem) the lowest (fig. 1). It has been shown that the auxin of Macrocystis is indole acetic acid, or a substance closely related to it, rather than auxin-a or -b.

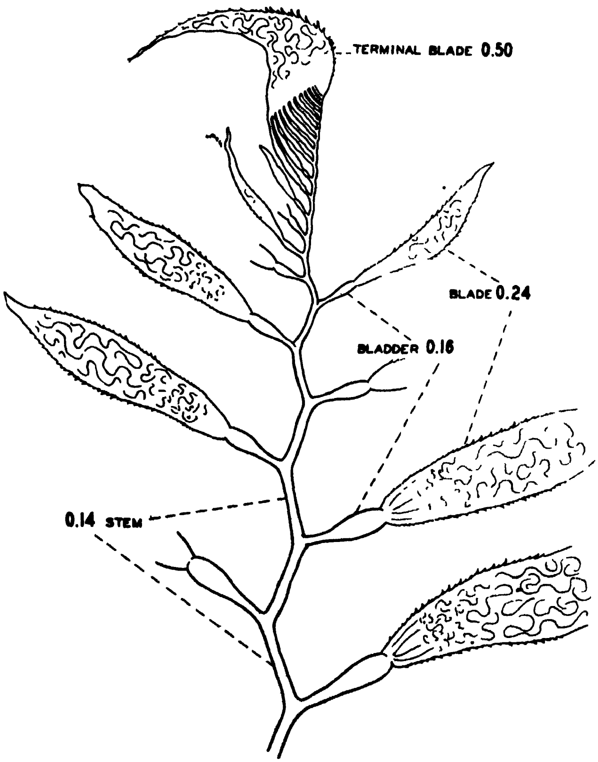


FIG. 1. Apical part of the thallus of *Macrocystis pyrifera* with figures indicating the distribution of auxin in gamma equivalents of indole acetic acid per kilogram fresh weight.

THE CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

LITERATURE CITED

1. ARVELT, K. Handbuch der physikalisch-chemischen Technik. F. Erdke, Stuttgart. 1923.
2. DOLK, H. E., and THIMANN, K. V. Studies on the growth hormone of plants. I. Proc. Nat. Acad. Sci. **18**: 30–46. 1932.
3. DU BUY, H. G., and OLSON, R. A. The presence of growth regulators during the early development of Fucus. Amer. Jour. Bot. **24**: 609–611. 1937.
4. HEYN, A. N. J. The chemical nature of some growth hormones as determined by the diffusion method. Proc. Kon. Akad. Wetensch. Amsterdam **38**: 1074–1081. 1935.
5. KÖGL, F. Wirkstoffprinzip und Pflanzenwachstum. Naturwiss. **25**: 465–470. 1937.
6. ————, ERXLEBEN, H., and HAAGEN-SMIT, A. J. Über die Isolierung der Auxine a und b aus pflanzlichen Materialen. Zeitschr. physiol. Chem. **225**: 215–229. 1934.
7. ————, HAAGEN-SMIT, A. J., and ERXLEBEN, H. Über ein neues Auxin (Hetero-auxin) aus Harn. Zeitschr. physiol. Chem. **228**: 90–112. 1934.
8. ————, and KOSTERMANS, D. G. F. R. Hetero-auxin als Stoffwechselprodukt niederer pflanzlichen Organismen. Isolierung aus Hefe. Zeitschr. physiol. Chem. **228**: 113–121. 1934.
9. KOSTERMAN, D. G. F. R. Over hetero-auxine Thesis, Utrecht. 1935.
10. OLTMANNS, FR. Morphologie und Biologie der Algen II. Fischer, Jena. 1922.
11. SETCHELL, W. A., and GARDNER, N. L. The marine algae of the Pacific Coast of North America. Univ. California Public. 8 (3). 1925.
12. VAN DER WEIJ, H. G. On the occurrence of growth substances in marine algae. Proc. Kon. Akad. Wetensch. Amsterdam. **36**: 759–760. 1933.
13. VAN OVERBEEK, J. "Laziness" in maize due to abnormal distribution of growth hormone. Jour. Hered. **29**: 339–341. 1938.
14. ————. A simplified method for auxin extraction. Proc. Nat. Acad. Sci. **24**: 42–46. 1938.
15. ————. Auxin distribution in seedlings and its bearing on the problem of bud inhibition. Bot. Gaz. **100**: 133–166. 1938.
16. ————, and BONNER, J. Auxin in isolated roots growing *in vitro*. Proc. Nat. Acad. Sci. **24**: 260–264. 1938.
17. WENT, F. W. Wuchsstoff und Wachstum. Rec. trav. bot. néerl. **25**: 1–116. 1929.
18. ————, and THIMANN, K. V. Phytohormones. Macmillan, New York. 1937.

SPIRAL SYSTEMS IN THE ORGANIZATION OF LIVING MATERIAL

W. D. FRANCIS

(WITH FIVE FIGURES)

Introduction

Since the invention of the microscope the problem of the structure of protoplasm has maintained a very prominent place in the study of life. Many investigators have hoped that the discovery of a mechanical structure in living material would yield some explanation of the remarkable functions connected with life. The colloidal concept of living material contributed much to the understanding of life processes, particularly in connection with surface reactions. On the other hand, the colloidal concept in so far as it is contrasted in a structural sense with the crystalloidal concept lent little or no support to the microscopist in his search for organized structure in living material. The recognition that the intricate structures and coordinated functions of organisms are compatible only with an orderly arrangement of living material brought the mind back to some such structure as crystals exhibit (**5**, **14**). Thus arose the present conception of protoplasmic constitution as being largely liquid crystalline.

Plant and animal protoplasm are generally assumed to be fundamentally similar. One basis of this assumption is that both kinds of protoplasm exhibit the general phenomena of life. Another basis for the assumption is the difficulty of differentiating whole groups of lower organisms as distinctively plant or animal in character. From these considerations one could expect that a definite structure which is found to be fundamental in the protoplasm of one species may be common to the protoplasm of all species.

Discussion

THE SPIRAL STRUCTURE OF CHROMOSOMES AND CYTOPLASM

The spiral structure of chromosomes and their finer deeply staining filaments or chromonemata has been established by recent work (**7**).

It has been remarked by SCARTH (**12**) that there is no occasion to suppose that the cell is ever without a skeleton of organized and probably orientated elements. The spiral structure of cytoplasm was observed by the writer (**6**) in a photomicrograph by SEIFRIZ (**13**) of the quiescent protoplasm of a slime mold.

One very readily and unconsciously assumes that microscopic vision is similar to ordinary vision with the unaided eye. As an example we may take the stamen hairs of *Rhoeo discolor,* which are sufficiently fine and transparent to be examined with the best immersion lenses. When the hairs are

mounted in water and examined under the microscope, the striated cellulose wall on the upper side comes into view and can be accurately resolved. Next the protoplasm within the hair cells can be focussed and seen clearly at any desired level. Finally, the cellulose wall on the lower side can be brought into view and its fine structure studied. It is to be remarked, however, that as the focus is altered either downwards or upwards the previous upper or lower field of view disappears. Thus microscopic vision at any one time is restricted to one plane which is roughly at right angles to the optical axis of the microscope. The higher the numerical aperture of the lens, and consequently the higher its resolving power, the more restricted is its field towards the plane of its sharpest focus. This peculiarity of microscopic vision accounts for the circumstance that insufficient attention has been given to structures which are beyond the field of view, but which are parts of the structures within the field of view. Gross structures such as cell walls are generally readily correlated with their parts, which are outside the field of view, by focussing. Very fine structures such as disperse aggregations and spirals, however, which are partly inside and partly outside the field of view, are often difficult to perceive and interpret. It is particularly pertinent to observe that the filament which composes the spiral, or helix, which we are discussing, never lies in one plane.

The granular appearance of protoplasm which is so often presented to the observer by the microscope is evidently due in great measure to optical sections through a spirally arranged disperse phase. The apparent granules are often round or slightly elongated images provided by spirally wound filaments as they are traversed crosswise or obliquely by the optical section provided by the microscope. Figure 1 is a diagram of a longitudinal section of a spiral. The diagram shows that a spiral in the position indicated is represented by two rows of dots, the dots of one row being placed alternately to the dots of the other row.

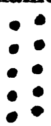


FIG. 1. Diagram of longitudinal section through a spiral. The spirally coiled filament appears as two rows of dots. The dots of one row alternate with the dots of the other row. When reversals in direction of the spirals occur, as in the spirals of chromosomes and cytoplasm, the pattern of dots is more irregular.

The limitation of microscopic resolution to sharply defined optical sections is strikingly revealed by the photomicrographs in figures 2 and 3. In parts of both of these photomicrographs fine protoplasmic connections are shown traversing the cellulose walls.

The advantage of photomicrography is that details which are invisible to the eye in the ordinary routine of microscopical observation are often shown up in a photomicrograph. Two examples will be given. Because of their recognized advantages, apochromatic lenses were used. On one occasion the writer photographed a precipitate of ferrous hydroxide which was mounted

on a slide in Canada balsam. The resulting plates showed long straight lines consisting of crystal edges and elongated crystals. These straight lines could not be found in the Canada-balsam preparation by visual observation. Later similar straight lines were detected by visual observation in preparations of the same material mounted in water. Evidently differences in the refractive properties of the mounted material and the mounting medium, which were insufficient to be detected by the eye, were registered on the photographic plate.

On another occasion a mounted preparation of iron rust was photographed under the microscope. The resulting picture clearly showed spiral structure in the rust. The writer had previously examined iron rust on numerous occasions without having observed its spiral structure. After it could be revealed by photographs, the spiral structure was detected in iron rust by ordinary visual methods with the microscope.

SEIFRIZ's photomicrograph (13) showing spiral structure in protoplasm led the writer to make photomicrographs of living protoplasm. The epidermis of the bulb scale of the onion (*Allium cepa*), freshly mounted in tap water, was the subject chosen. Figures 2 and 3 are reproductions of some of the photomicrographs. The photomicrographs in this paper are of the "silver skin" type of onion.

The photomicrographs show spiral structure in the protoplasm, in the oil bodies suspended in the protoplasm, and in parts of the cellulose walls. The oil bodies were seen in the protoplasm before the photographs were taken. They are often irregular, angular, or rounded in shape and strongly light-refracting. The cellulose walls in the "silver skin" variety were also strongly light-refracting. This light-refracting property which is rendered by the more pale color differentiates the oil bodies and cell walls from the background of the protoplasm in the photomicrographs. Within the oil bodies as well as in the protoplasm are dark spiral filaments. From visual studies and from photomicrographs in which the material composition of the structures is known one may interpret these dark spirals as protein. For example, the darker portions of the nucleus both in visual observations and in photomicrographs consist of the chromonemata which are composed of protein. KUWADA and NAKAMURA's photomicrograph (8) of a dividing nucleus in a living stamen hair of Tradescantia shows the chromonemata as dark twisted filaments. Confirmation of the interpretation of the dark spirals as protein is provided by ANDREWS (2), who found that the oil bodies of plants consist of oil and a protein base. .

The distribution and character of the oil of the onion and allied species is outlined by MOLISCH (11). In common with many other authors he refers to the oil as allyl sulphide. CZAPEK (3) remarks that allyl-propyl disulphide appears to be an important constituent of the oil of the onion. From the

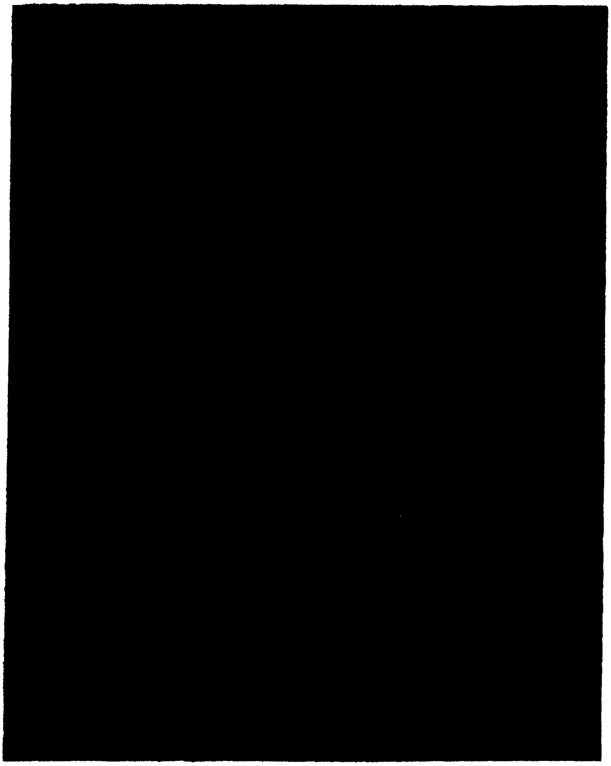


FIG. 2. Unretouched photomicrograph of living protoplasm of epidermis of bulb scale of onion. Apochromat 2 mm. n.a.1.33. × 1250. The pale light-refracting areas in the cells represent onion oil. This is shown as spirals, or sections of spirals, often surrounding dark areas evidently of protein. The spiral character of the dark proteinaceous parts is well shown on the left of the lowermost cell where the spiral protein structures are invested by the pale light-refracting spirally arranged oil. The cell wall in the left upper corner is shown as coiled in two strands consisting of left-hand spirals. The appearance of the cell wall here is suggestive of a cord composed of two strands. Indications of left-hand spiral structure are also shown in the cell wall towards the lower part of the photograph. A few fine protoplasmic connections are shown piercing the cell wall in the upper part of the photograph. The discontinuity of the cell wall on the left is evidently caused by the optical section traversing coarse perforations in the cell wall.

location and description of onion oil as outlined by MOLISCH and others it is evident that it is the strongly light-refracting, spirally arranged material appearing in variously shaped bodies in the photomicrographs presented here (figs. 2, 3).

According to LEPESCHKIN (9), protein and lipoids are the principal constituents of protoplasm, of its dispersion medium, and of its disperse phases. He defines lipoids as subtances which are found in organisms and

which are distinguished by their solubility in ether, benzol, chloroform, oil, and partly in alcohol and their insolubility in water. According to this definition onion oil would be included with lipoids.

In accordance with this broad definition of lipoids the writer's photomicrographs provide a picture of the coarse disperse phases of the principal materials of protoplasm; namely, the protein and the lipoid. In LEPESCH-

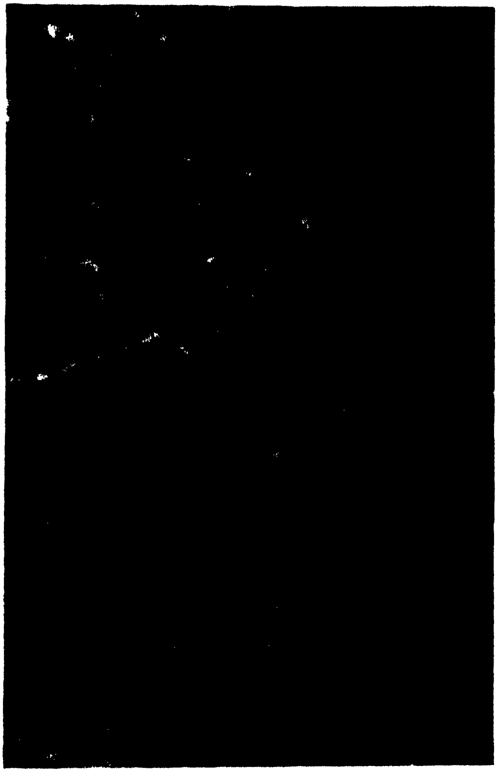


FIG. 3. Unretouched photomicrograph of living protoplasm of epidermis of bulb scale of onion. Apochromat 2 mm. n.a.1.33. Enlarged to ×1143 from original photograph, ×750. The coarse and fine dark twisted spirals in different parts of the photograph are evidently proteinaceous. The pale, light-refracting portions investing the dark spirals represent onion oil; their spiral formation is more evident in the upper part of the photograph. In the upper left portion there are systems of fine and coarse spirals connected by fine anastomoses. The upper part of the photograph is strongly suggestive of protoplasmic organization on the basis of interconnecting spiral systems of descending orders of size. Fine protoplasmic connections are shown perforating the cell wall which passes across the middle of the photograph. It is to be observed that the protoplasmic connections shown in this figure and in figure 2 are dark colored, indicating their proteinaceous character.

KIN's photomicrographs (9, fig. 16, 18) of living protoplasm, alternately dark and light areas are shown. These may represent areas in which protein and lipoids respectively are dominant.

In parts of the writer's photomicrographs the coarse disperse phases of the protoplasm are shown as a lining in contact with the cell wall. In these places the outer layer of the protoplasm would present a surface composed of alternating spiral coils of protein and lipoid. See for example the lower side of the cell wall in figure 3. This photomicrographic feature of the outer layer of protoplasm is significant in connection with NATHANSOHN's conception of the "plasma membrane" as possessing a mosaic structure consisting of proteins alternating with lipoids (9, p. 147.)

In the upper left-hand side of figure 3 there are indications of an orderly arrangement of the spiral systems. Several large spirals are shown passing downwards from near the top of the photograph. One of these spiral systems, the one on the left, is diagrammatically represented in figure 4. It is to be observed that the larger spiral consists of a filament which is wound in a spiral of a lower and much finer order than that of the large coils. Further, it is observable in the upper left-hand

FIG. 4. Diagram of a spiral system shown on upper left of figure 3. The larger spiral coils are composed of a filament which is wound into a spiral of a lower order of size.

side of figure 3 that the large spiral systems, such as those shown diagrammatically in figure 4, are connected with adjacent spiral systems by fine connecting strands (fig. 5). These fine connecting strands may be parts of spirals which are intertwined with the spirals which they appear to connect. The connecting strands are apparently similar to the

FIG. 5. Diagram of portions of two spiral systems, upper and lower, from upper left side of figure 3. The two spiral systems are connected by two anastomoses.

anastomoses of the chromatinic reticulum of nuclei.

The writer has frequently noticed while examining living protoplasm that spirals appear to be visible with lenses of ascending magnifications such as with 16, 4, and 2 mm. lenses. This suggests that there are spirals of ascending and decending orders of size. This observation is confirmed to some extent by figure 3 which shows spirals of two orders of size.

Spirals of ascending and descending orders of size may be the primordial structural basis of the coordination of functions in living systems.

INDICATIONS OF SPIRAL STRUCTURE IN POTATO STARCH GRAINS AND CRYSTALS OF CANE SUGAR

ALSBERG (1) apparently quoting MEYER, HOPFF and MARK, states that starch-chain molecules are perhaps spirally wound. He also quotes NÄGELI as reporting spirally twisted starch granules.

The writer has examined the starch grains of the potato by bringing a glass slide into contact with a freshly cut potato and allowing the starchy film to dry in air for a few days. In preparations of this kind the material of the starch grains, which were sufficiently transparent, showed series of curves and crescents arranged in such a way as to indicate that they formed parts of closely wound spirals.

Crystals of cane sugar were spread on glass slides, which were slightly moistened with water, and allowed to dry. Some of the crystals which could be examined microscopically showed dark internal patterns indicative of denser areas of spiral formation.

Spiral structure in parts of the cellulose walls of the onion has already been mentioned (see also fig. 2). According to FARR (4) cellulose fibrils arranged in cross spirals have become familiar objects in published illustrations of the swollen membranes of cotton fibres.

SPIRAL SYSTEMS AND INTEGRATION

The widespread occurrence of spiral systems in living protoplasm leads one to inquire if they can be correlated with any functions of the organism. The short spirals such as those in chromosomes and cytoplasm are suggestive of the metallic spirals used in physics as models of short solenoids. When an electric current is passed through these short metallic spirals they exhibit a characteristic magnetic field similar to that of bar magnets. It is possible that in living systems the proteinaceous spirals may possess magnetic fields comparable to those of short solenoids and bar magnets.

The repulsive and attractive movements of chromosomes in mitosis and meiosis are strongly suggestive of magnetic effects. In mitosis the repulsive effects may be produced by electric currents flowing in the same direction in similarly coiled chromatids. In meiosis the attractive effects which produce pairing of homologous chromosomes may be brought about by electric currents flowing in oppositely coiled chromosomes. The work of LUND and his co-workers (**10**), for example, shows that there are electric currents in living organisms. LUND and his associates have also shown that these electric currents are dependent upon cell respiration. According to WARBURG (**15**), cell respiration is a capillary-chemical process which takes place on the surface of the solid cell constituents. As the spirals in protoplasm constitute its principal disperse phase, it is to be expected that the surfaces of the spirals would be the seat of cell respiration, and consequently the loci of electric potentials. Another significant fact is the participation of iron compounds (haemin compounds) in cell respiration. It is generally recognized that the metals themselves are electric conductors of the first class.

In view of the preceding considerations it is suggested that the spirals in protoplasm may be activated by electric currents and possess magnetic fields

comparable to those of bar magnets. If this is so, the positions of the spirals in space and their relationship to each other would be determined by lines of force connected with the spirals themselves. Such an arrangement would represent a dynamic system with considerable possibilities for the fundamental explanation of structures and functions of organisms on an electromagnetic basis.

Integration or the harmonious functioning of the organism as a unit may depend upon protoplasmic spiral systems as the loci of electric potentials and the seat of electromagnetic fields.

Summary

In extending observations on the spiral structure of protoplasm, photomicrographs were taken of the living protoplasm in the epidermis of the bulb scale of the onion. The photomicrographs show spiral structure in the protoplasm, in the oil bodies suspended in the protoplasm, and in parts of the cellulose walls.

Spiral structure in cellulose walls and oil bodies and indications of spiral structure in potato starch grains and crystals of cane sugar suggest that this type of structure may also be characteristic of protoplasmic products.

In parts of the photomicrographs there are indications of an orderly arrangement of the spirals into systems. The filament composing some of the spirals is shown to be coiled into spirals of two orders of size.

Spirals of ascending and descending orders of size may be the primordial structural basis of the coordination of functions in living systems.

It is suggested that the proteinaceous spirals in protoplasm may be activated by electric currents and possess magnetic fields comparable to those of short solenoids and bar magnets. If this is so, the position of the spirals in space and their relationship to each other would be determined by lines of force connected with the spirals themselves. Such an arrangement would represent a dynamic system with considerable possibilities for the fundamental explanation of structures and functions of organisms on an electromagnetic basis.

Integration or the harmonious functioning of the organism as a unit may depend upon protoplasmic spiral systems as the loci of electric potentials and the seat of electromagnetic fields.

Botanic Gardens
Brisbane, Australia

LITERATURE CITED

1. Alsberg, C. L. Structure of the starch granule. Plant Physiol. 13: 295–330. 1938. (See p. 307.)
2. Andrews, F. M. Oil bodies in plants. Abstr. and Titles of Papers, 15th Ann. Meeting, Amer. Society Plant Physiol., p. 12, 1938.

3. CZAPEK, F. Biochemie der Pflanzen, Auf. 3, Bd. 3, p. 190. Fischer, Jena. 1925.
4. FARR, W. K. The microscopic structure of plant cell membranes in relation to the micellar hypothesis. Jour. Phys. Chem. **42**: 1113–1124. 1938.
5. FRANCIS, W. D. The crystalline character of living material. Science n. s. **87**: 16. 1938.
6. ———. The spiral structure of protoplasm. Science n. s. **89**: 127. 1939.
7. KOSHY, T. K. Number and behaviour of chromosomes in *Aloe litoralis*. Ann. Bot. n. s. **1**: 52–58. 1937.
8. KUWADA, Y., and NAKAMURA, T. Behaviour of chromonemata in mitosis. VI. Metaphasic and anaphasic longitudinal split of chromosomes in the homotype division in pollen mother cells in *Tradescantia reflexa*. Cytologia **6**: 314–319. 1935.
9. LEPESCHKIN, W. W. Kolloidchemie des Protoplasmas. p. 146. J. Springer, Berlin. 1924.
10. LUND, E. J., and BUSH, M. Electric correlation potentials in the leaf of *Bryophyllum*. Plant Physiol. **5**: 491–508. 1930.
11. MOLISCH, H. Mikrochemie der Pflanze, 2 Auf. p. 139. Fischer, Jena. 1921.
12. SCARTH, G. W. The structural organization of plant protoplasm in the light of micrurgy. Protoplasma **2**: 189–205. 1927. (See p. 194.)
13. SEIFRIZ, W. Protoplasm. fig. 119. McGraw-Hill, New York. 1936.
14. ———. Recent contributions to the theory of protoplasmic structure. Science n. s. **88**: 21–25. 1938.
15. WARBURG, O. Physikalische Chemie der Zellatmung. Biochem. Zeitschr. **119**: 134–166. 1921.

RELATION BETWEEN QUANTITY OF CHLOROPHYLL AND CAPACITY FOR PHOTOSYNTHESIS

ROBERT EMERSON, LOWELL GREEN, AND J. LEYDEN WEBB

(WITH ONE FIGURE)

Seeking evidence as to the number of chlorophyll molecules concerned in the reduction of one molecule of carbon dioxide, EMERSON and ARNOLD (3) measured the maximum amount of carbon dioxide which could be reduced by a measured quantity of cells, under conditions designed to make photosynthesis dependent only on the amount of chlorophyll present, and independent of other factors, both internal and external. The limiting effect of chemical processes in the cell under saturating external conditions was avoided by illuminating with extremely short flashes of light (about 10^{-5} seconds) and allowing sufficient dark time (about one-tenth of a second) between flashes so that the chemical processes or Blackman reaction could run to completion between light flashes. The intensity of the flashes was so great that higher intensities resulted in no increase in carbon dioxide assimilation. With light flashes as short as 10^{-5} sec., it was believed impossible for a significant number of individual chlorophyll molecules to act more than once per flash in the reduction of carbon dioxide. Under these conditions it was anticipated that chlorophyll would be the only factor limiting the amount of carbon dioxide reduced per flash. The number of chlorophyll molecules should limit the energy available for carbon dioxide reduction after each flash. The ratio (ρ) of number of chlorophyll molecules present to number of carbon dioxide molecules reduced per flash was found to remain approximately constant, even when the chlorophyll content per unit volume of cells was varied several fold. Unexpectedly high values were obtained for ρ, showing that the cells contained two or three thousand times as much chlorophyll as the maximum amount of carbon dioxide which they could reduce per flash of light.

Calculations made by GAFFRON and WOHL (7) show that essentially the same relationship prevails in continuous light. They compared the maximum rate of carbon dioxide reduction with the chlorophyll content for a variety of material, including leaves as well as suspensions of algal cells. Taking the average time required for the completion of the Blackman reaction in Chlorella from the results of EMERSON and ARNOLD, they found that assimilating cells in general contain over two thousand times as much chlorophyll as seems necessary to account for the highest observed rates of photosynthesis.

The measurements of EMERSON and ARNOLD were made only with *Chlorella pyrenoidosa,* but ARNOLD and KOHN (1) later reported measure-

ments with six species, representing four phyla. Their results, and the calculations of GAFFRON and WOHL, have led to the proposal that chlorophyll exists in the cell in some sort of units, each containing some 2000 chlorophyll molecules, such that when a quantum of light is absorbed by any member of a particular unit, the energy may become available for the reduction of a carbon dioxide molecule associated with the unit (*cf.* ARNOLD and KOHN, **1**; GAFFRON and WOHL, **7**; WOHL, **8**, **9**, **10**). Some of ARNOLD and KOHN's values for ρ run up to four and five thousand, suggesting that ρ may be subject to some variation. Nevertheless, they regard its relative constancy as evidence for the existence of chlorophyll units. Therefore it seemed to the writers important to find within what limits ρ might vary in a single species, *Chlorella pyrenoidosa*. The experiments we report here show a much greater range in the value of ρ than had been indicated previously, so that hypotheses formulated on the basis of the supposed constancy of ρ may not be tenable. The existence and significance of the chlorophyll unit is now the subject of active discussion (*cf.* WOHL, **8**, **9**, **10**; also FRANCK and HERZFELD, **5**; FRANCK and TELLER, **6**) on which our results appear to have some bearing.

Experimental

Except for the use of a culture medium saturated with five per cent. carbon dioxide instead of carbonate-bicarbonate mixture as suspending fluid for the cells, our technique was the same as EMERSON and ARNOLD's. In view of the results reported by EMERSON and GREEN (**4**), who compared the behavior of photosynthesis in carbonate mixtures and in phosphate buffers, we thought it possible that the phenomena in flashing light might depend in some respect on the pH or on the method of carbon dioxide provision. In a culture medium saturated with five per cent. carbon dioxide, photosynthesis is unaffected by considerable variations in both pH and carbon dioxide concentration.

The carbon dioxide assimilation during flashing light is of the same order of magnitude as the respiration, so the correction applied for respiration is of the utmost importance. Respiration measurements were made in darkness, between the exposures to flashing light. Separate experiments on respiration indicated that the small amount of assimilation during flashing light was without measurable influence on the subsequent rate of respiration. As in all measurements of photosynthesis, it remains possible that the respiration is higher during illumination so that the use of a subsequent respiration measurement to compute photosynthesis may lead to a value lower than that actually attained by the organism.

The chlorophyll content was determined by extracting aliquot samples of cells with hot methyl alcohol, and measuring the extinction spectrophotometrically for the Neon line 6598 Å. The same method was used by EMER-

SON and ARNOLD, and by ARNOLD and KOHN. It has been standardized with weighed samples of Chlorella chlorophyll (EMERSON and ARNOLD, 3) and with chlorophyll prepared from higher plants (ARNOLD and KOHN, 1) and the values obtained were practically identical.

The accuracy of the method depends on several factors besides the precision of the standardization. Chief among these are the completeness of extraction of the chlorophyll from the cell samples, the freedom of the extract from other substances which absorb appreciably at 6598 Å, and the uniformity of the ratio of the two chlorophyll components. While we cannot state quantitatively how closely these requirements are fulfilled, it seems conservative to say that the error in the analyses is probably less than ten per cent. Since we are concerned here with variations of several fold in the value of ρ, such an error in the chlorophyll determinations would not alter our conclusions.

Most of the photosynthesis measurements were made at 25° C., with a frequency of 20 flashes per second, making the dark time between flashes 0.05 sec. According to EMERSON and ARNOLD, at 25° this dark time is sufficient for the completion of the dark processes after each flash. Some of our experiments suggest that cells from young cultures grown over bright light may require a longer dark period. If this proves to be the case, then the

TABLE I

THE RATIO ρ IS SHOWN FOR CULTURES OF VARIOUS AGES, GROWN OVER THREE DIFFERENT SOURCES OF ILLUMINATION. ALL CULTURES WERE GROWN AT 17° C. ± 1°. MEASUREMENTS OF ρ WERE MADE AT 25° C.

CURVE	LIGHTING CONDITIONS FOR GROWTH OF CULTURES	AGE AT TIME OF HARVESTING	DENSITY AT TIME OF HARVESTING	ρ, $\frac{\text{MOLES OF CHLOROPHYLL}}{\text{MOLES } CO_2 \text{ REDUCED PER FLASH}}$
		days	*mm.³ per ml. of culture*	
A	Four forty-watt incandescent lamps 10 cm. from culture flasks, and Corning "Noviol C" filter, transmitting only wave lengths longer than 480 mμ	2 4 8	0.1 0.5 1.6	3,750 6,180 11,120
B	Four forty-watt incandescent lamps 10 cm. from culture flasks, no filter.	4 9 17	0.6 1.6 2.2	3,920 6,850 9,720
C	Single forty-watt incandescent lamp, 15 cm. from culture flasks.	15 21 29	0.2 0.5 0.7	6,650 11,000 14,500

values of ρ shown in table I may have to be reduced somewhat for the younger cultures.

The ratio ρ was determined for cells from cultures grown for different periods of time and with different conditions of illumination. In all cases it was found to increase with increasing age of the cultures, but the rate of increase and the value at any given age depended on the illumination used for growing the cultures. Much experimental work has been done on the influence of wave length and intensity of light on the changes in chlorophyll content and photosynthetic capacity with age of culture. The action of these factors is obscured by the increasing selective absorption of light by the cells in a growing culture. This is minimal in a young culture which contains so few cells that light passes through the suspension almost unchanged. But as the culture grows older and increases in density, certain wave lengths are absorbed more strongly than others, and the light which has passed through the peripheral parts of the suspension reaches the cells in the interior greatly diminished in intensity and changed in wave length distribution. The cells are circulated by shaking the cultures at regular intervals, and by the slow stream of five per cent. carbon dioxide in air which bubbles through continuously, so that all are exposed from time to time to the entire range of illumination prevailing within the culture. The changing environment inevitably associated with this simple technique renders it difficult to separate and analyze the influence of the factors in question. While it is clear that the age of the culture, the wave length distribution, and light intensity used for growing the culture all have profound effects on the photosynthetic apparatus, a separation of these effects probably requires more elaborate culture technique. Consequently the present paper deals only with variations in ρ, without attempting to trace the influence of culture conditions on the more fundamental internal factors upon which ρ probably depends.

Figure 1 shows three curves for the value of ρ plotted against age of culture for cultures grown under different conditions of illumination. The conditions of illumination for the three curves, as well as the density of each culture at the time of harvest, the age, and the value of ρ, are given in table I. The lighting used for the cultures for curves A and B was identical except for the use of a filter[1] for the "A" cultures which transmitted only wave lengths longer than about 480 mμ. The cultures for curve A were therefore deprived of blue and violet light, while the cultures for curve B were exposed to the full spectrum of the incandescent lamps. Comparison of the densities for equal ages of the A and B series in table I shows that the removal of the blue and violet leaves the rate of growth practically unchanged, although for corresponding densities ρ is always higher for the cells grown without blue and violet light.

[1] Corning "Noviol C" filter.

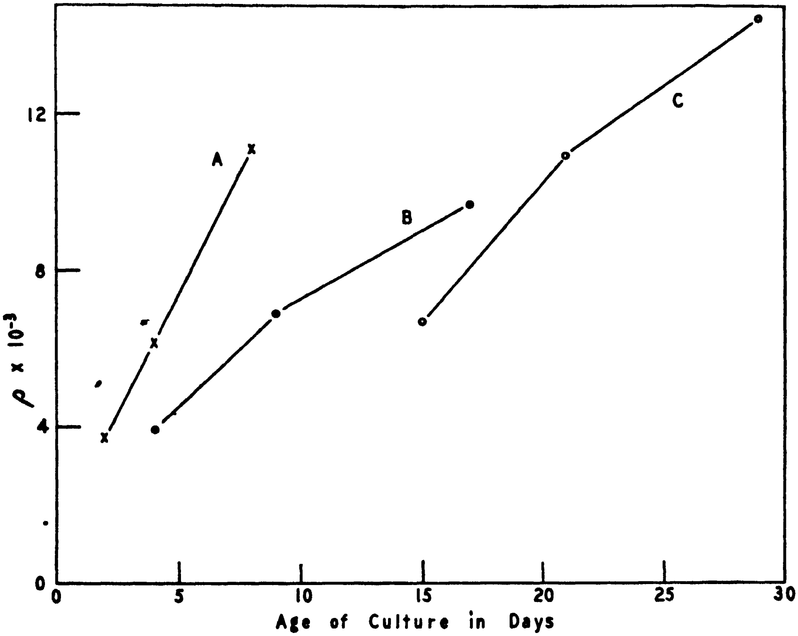


FIG. 1. Three curves showing the influence of age of culture on the value of ρ. The illumination for growing the cultures for curve B was from four forty-watt incandescent lamps spaced close together in a square, and 10 cm. distant from the bottoms of the culture flasks. The same lighting arrangement was used for the cultures for curve A, but in this case the light was passed through a glass color filter transmitting only wave lengths longer than about 480 mμ. The cultures for curve C were grown over a single 40-watt incandescent lamp at a distance of 15 cm.

Curve C, made with a series of cultures grown at a very low light intensity, shows higher values of ρ for corresponding culture densities, or lower values for corresponding ages.

Discussion

Our results indicate that the value of ρ regularly increases with the age of the culture, and is influenced by the lighting conditions used for growth. None of the values reported here are as low as the highest values of EMERSON and ARNOLD (3), but no attempt was made to duplicate their culture conditions. Their primary purpose was to obtain a wide range of chlorophyll concentrations. This they achieved by growing cultures over neon and mercury sign-lighting tubes. The approximate constancy of ρ over a wide range of chlorophyll concentrations seems remarkable, but may be attributable in part to the fact that their cultures were regularly harvested as soon as they had reached a density which provided adequate amounts of material for the measurements, without reference to age.

It might be supposed that the increase in ρ with age of culture indicates an increasing proportion of inactive or moribund cells which still retain their cholorophyll. This would be consistent with the opinion of ARNOLD and KOHN that in all active cells there exists a chlorophyll unit of approximately constant size. There is no reason however, for thinking that our cultures contained increasing quantities of inactive cells. The decline in growth rate with age indicated by the figures for culture density in table I is no more than would be anticipated from the increasing competition for light and nutrients with increasing population density. Differential centrifuging failed to reveal the presence of more and less active fractions among the cell population. We regard it as unlikely that there are appreciable quantities of inactive cells present in cultures during periods of rapid growth, although table I shows that this rapid growth may be accompanied by a sharp increase in the value of ρ.

It seems more probable that a chlorophyll unit within the meaning of ARNOLD and KOHN does not exist, and that the large amount of chlorophyll present in comparison with the capacity to reduce carbon dioxide must be explained in other terms. FRANCK and HERZFELD (5), and FRANCK and TELLER (6) have found it difficult to reconcile the existence of such a unit with modern concepts concerning energy transference between large molecules. EMERSON (2) has suggested that the amount of carbon dioxide which can be reduced per flash of light may not depend upon the amount of chlorophyll, but may be a measure of some other substance taking part in photosynthesis. For example, it is possible that assimilating cells contain a limited amount of some substance with which carbon dioxide must combine before it can undergo reduction. Carbon dioxide saturation in flashing light would then be a measure of the full capacity of this substance for combining with carbon dioxide. The fact that ρ is always large would mean that the amount of chlorophyll always greatly exceeds the amount of this proposed substance. Other catalysts or enzymes present in small amount could also account for the observed phenomena, provided they could set a fixed limit to the yield per flash under saturating conditions. The best obtainable yield per flash appears to be always far short of what might be expected from the amount of chlorophyll present.

Summary

During photosynthesis in flashing light under optimum external conditions, the ratio of moles of chlorophyll present to moles of carbon dioxide reduced per flash is not a constant in *Chlorella pyrenoidosa* cells, but depends on conditions of previous growth, increasing sharply with age of culture and varying with color and intensity of culture illumination. Thus the maximum amount of carbon dioxide reducible per flash is not directly

related to the amount of chlorophyll but depends on some other internal factor.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

LITERATURE CITED

1. ARNOLD, WILLIAM, and KOHN, HENRY I. The Chlorophyll Unit in Photosynthesis. Jour. Gen Physiol. **18**: 109–112. 1934.
2. EMERSON, ROBERT. Photosynthesis. Ann. Rev. Biochem. **6**: 535–556. 1937.
3. ———, and ARNOLD, WILLIAM. The photochemical reaction in photosynthesis. Jour. Gen. Physiol. **16**: 191–205. 1932.
4. ———, and GREEN, LOWELL. Effect of hydrogen-ion concentration on Chlorella photosynthesis. Plant Physiol. **13**: 157–168. 1938.
5. FRANCK, JAMES, and HERZFELD, K. F. An attempted theory of photosynthesis. Jour. Chem. Phys. **5**: 237–251. 1937.
6. ———, and TELLER, E. Migration and photochemical action of excitation energy in crystals. Jour. Chem. Phys. **6**: 861–872. 1938.
7. GAFFRON, HANS, and WOHL, KURT. Zur Theorie der Assimilation. Naturwiss. **24**: 81–107. 1936.
8. WOHL, KURT. Zur Energiebilanz der Kohlensäureassimilation. Zeitschr. physikal. Chem. (B) **31**: 152–156. 1935.
9. ———. Zur Theorie der Assimilation, I. Die Theorie der Assimilationseinheit. Zeitschr. physikal. Chem. (B) **37**: 105–121. 1937.
10. ———. Zur Theorie der Assimilation, IV. Zum Mechanismus der Assimilationseinheit. Zeitschr. physikal. Chem. (B) **37**: 186–208. 1937.

EXPERIMENTS ON PHOTOPERIOD IN RELATION TO THE VEGETATIVE GROWTH OF PLANTS[1]

JAMES BONNER

Introduction

It has long been known that relative length of day and night effects the vegetative as well as the reproductive activity of plants. Since the appearance of the paper of GARNER and ALLARD (7) which first dealt with this subject, abundant factual material has accumulated relating to the influence of photoperiod on such varied processes as shoot elongation, leaf size, accumulation of dry weight and root growth (6, 10). It has been frequently, although not invariably, found that vegetative growth as measured by one or more of these criteria, is greater in long days than in short days irrespective of the reproductive behavior of the species in question. The promotive effect of long daily photoperiods on vegetative growth does not appear to be directly attributable to increased duration of CO_2 assimilation (11). Plants grown under conditions of long day made up of a short photoperiod of natural light supplemented by light of exceedingly low intensity exhibit the vegetative vigor typical of plants on a long day rather than that of plants on a short day regime (13).

It is known that the reproductive response of photoperiod sensitive plants is to be attributed to the production of a specific hormone (or hormones) in the leaves under conditions of suitable day length (8, 9). The production of this particular hormone or hormones would appear to depend on the length of day to which the leaves are subjected. It seems logical to inquire in how far the vegetative responses of plants to varying photoperiods are also attributable to the influence of day length on the production of the several hormones which regulate vegetative growth. In the present paper evidence will be presented to show that the production of vitamin B_1, a hormone for the growth of roots (1), is affected by the length of day to which the plant is subjected.

It is known that length of day exercises a profound effect on the extent of the root system in many species of plants. Thus WEAVER and HIMMEL (12) found that the root system is invariably more extensive under conditions of long photoperiod irrespective of whether the species of plant is long day, short day, or indeterminate with regard to flowering behavior. CRIST and STOUT (5) have also shown that the root system of plants grown under short photoperiod may be several times smaller than the root systems of similar plants grown under conditions of long photoperiod. These observa-

[1] Report of work done with the cooperation of the Works Progress Administration; Official Project no. 665–07–3–83, Work Project no. 9809.

tions suggest that the roots of plants grown under short photoperiod receive a limited amount of one or more of the substances required for root growth and supplied by the above ground portion of the plant.

Materials and methods

Plants were grown in the greenhouse in the manner previously described (**2**) in washed quartz sand contained in 2-gallon glazed crocks provided with drainage. They were supplied on alternate days with SHIVES R_2S_5 nutrient (including the minor elements Mn, Cu, Zn, B, Mo, and Fe) and the crocks flushed out with tap water on the intervening days. In each experiment one half of the plants were supplied with vitamin B_1 at the rate of 0.01 mg. per liter of nutrient solution. This concentration has been found in earlier experiments (**2**) to be non-toxic and promotive to the growth of many species of plants. Conditions of long photoperiod (18 to 20 hours) were maintained by means of supplementary illumination from Mazda lamps, approximately 80 foot-candles intensity at the leaf surface. Conditions of short photoperiod refer to a daily light period of 9 hours (8 A.M. to 5 P.M.).

In each experiment the vitamin B_1 content of the leaves (and in some cases of the roots) of the control and experimental plants was determined. This was done by means of the Phycomyces assay whose application to determination of vitamin B_1 in plant tissues has been described in detail in an earlier publication (**3**).

Experimental results

It is shown in table I that of the five species examined in the present experiments, every one contained less vitamin B_1 when cultured under a short photoperiod than when cultured under a long photoperiod. Of these spe-

TABLE I

SUMMARY OF DATA RELATIVE TO THE INFLUENCE OF PHOTOPERIOD ON VITAMIN B_1 CONTENT OF TOPS OF FIVE SPECIES OF PLANTS, GROWN IN SAND CULTURE AND ON LONG OR SHORT PHOTOPERIOD

SPECIES	FLOWERING PHOTOPERIODIC BEHAVIOR	VITAMIN B_1 CONTENT			
		SHORT PHOTOPERIOD		LONG PHOTOPERIOD	
		PER GRAM DRY WEIGHT	PER SHOOT	PER GRAM DRY WEIGHT	PER SHOOT
		γ	γ	γ	γ
Xanthium	Short day	3.4	1.72	5.2	4.20
Cosmos	" "	4.9	0.26	5.1	0.38
Brassica alba	Long day	4.85	0.31	6.0	0.54
Brassica nigra	" "	2.95	0.14	3.9	0.22
Lycopersicum	Indeterminate	5.3	2.86	6.5	6.78

cies, two were short day with respect to production of flower hormones (that is, were "short day" plants), two were long day with respect to the production of this factor, and one was indeterminate. Nevertheless, all might be termed "long day" with respect to the production of vitamin B_1. It appears then justifiable to advance the working hypothesis that the vegetative growth responses of plants to photoperiod may be attributable in part to the decreased production of vitamin B_1 under conditions of short day. Support of this hypothesis is supplied by the fact that plants whose vitamin B_1 content is low because of maintenance under conditions of short photoperiod, do in certain cases respond more strongly to added vitamin than do parallel plants maintained under conditions of long photoperiod (table II, III).

TABLE II

EFFECT OF PHOTOPERIOD AND VITAMIN B_1 ON THE GROWTH OF XANTHIUM IN SAND CULTURE

	Short photoperiod					Long photoperiod				
	Dry weight per plant		Vitamin B_1 content		Number of plants	Dry weight per plant		Vitamin B_1 content		Number of plants
	Tops	Roots	Tops	Roots		Tops	Roots	Tops	Roots	
	mg.	*mg.*	γ	γ		*mg.*	*mg.*	γ	γ	
Control	510	57	1.72	0.19	47	810	133	4.20	0.69	36
Added vitamin B_1	855	105	3.76	0.46	37	1000	195	7.20	1.40	41

TABLE III

EFFECT OF PHOTOPERIOD AND VITAMIN B_1 ON THE GROWTH OF BRASSICA PLANTS IN SAND CULTURE. PLANTS HARVESTED SIX WEEKS AFTER PLANTING

Species	Short photoperiod		Long photoperiod	
	Dry weight per plant	Vitamin B_1 content	Dry weight per plant	Vitamin B_1 content
	mg.	γ	*mg.*	γ
B. alba				
Control	64	0.31	90	0.54
Added vitamin B_1	290	2.25	193	3.05
B. nigra				
Control	48	0.14	57	0.22
Added vitamin B_1	175	1.01	136	0.87

Xanthium pennsylvanicum (cocklebur) is a short day plant and flowers if maintained under daily photoperiods shorter than 15.5 hours. In the present experiments, the seedling plants were maintained for 3 weeks under

conditions of long (18-hour) photoperiod. They were then divided into four equal lots and two lots transferred to conditions of a short (9-hour) photoperiod. One lot of those on each photoperiod received nutrient solution with added vitamin B_1; the other 2 lots received only nutrient solution. At the end of 4 weeks, when the plants were harvested, those maintained on long photoperiod were strictly vegetative while those maintained on short photoperiod possessed flowers and young fruits. Table II shows that of the control plants, those maintained under short photoperiod formed only 61 per cent. of the dry weight formed by those on long photoperiod, and that the dry weight of roots produced on short photoperiod was less than half that produced on long photoperiod. The plants maintained on short photoperiod and supplied with vitamin B_1, however, actually exceeded the long photoperiod control plants in total dry weight and produced 81 per cent. of the dry weight formed by the long photoperiod vitamin B_1 plants. The increased response to vitamin B_1 under conditions of short photoperiod is particularly striking in the case of the root system. The roots of the vitamin B_1 treated plants were almost 100 per cent. heavier than those of the control plants under conditions of short photoperiod and were only 44 per cent. heavier than the control roots under conditions of long photoperiod. Table II shows, then, that Xanthium plants on short photoperiod which contain relatively little vitamin B_1, respond more vigorously to addition of vitamin B_1 (with increased dry weight deposition) than do the plants on long photoperiod which contain relatively much vitamin B_1.

Brassica alba and *Brassica nigra* grown from unvernalized seed behave as long day plants. In the present experiments two lots of each species were maintained on long photoperiod and two lots of each species on short photoperiod. At the expiration of 6 weeks when the plants were harvested, those on long photoperiod were in full bloom, while those maintained continuously on short photoperiod were vegetative. Table III shows that with both species, less dry weight was accumulated (in the control series without vitamin B_1) under short photoperiod than was accumulated under long photoperiod. In each case also considerably less vitamin B_1 was found in the plants under short photoperiod than was found in the plants under long photoperiod. With both species the percentage increase in dry weight under the influence of vitamin B_1 is greater under short photoperiod than under long photoperiod.

It has been shown by ČAJLACHJAN and ŽDANOVA (4) that more auxin is produced by plants maintained under long photoperiods than by similar plants maintained under short photoperiods. In the present experiments it was found that Xanthium grown under long photoperiod contained considerable amounts of auxin,[2] but when grown under conditions of short

[2] Auxin determinations by DR. J. VAN OVERBEEK.

photoperiod it contained no appreciable amount of auxin. The production of auxin by the plant appears to be affected by photoperiod just as is the production of vitamin B_1. It is to be expected that not only the formation of auxin and vitamin B_1 but also the formation of other growth factors may be affected by the daily photoperiod to which the plant is subjected. Under the conditions of the present experiments it would appear that production of vitamin B_1 is a particularly limiting factor in the case of Xanthium and of Brassica grown under conditions of short photoperiod. In other cases, other factors may be even more greatly affected than vitamin B_1. This may be the case with Cosmos whose response to added vitamin B_1 is less under short day conditions than under conditions of long day (table IV) and with tomato which does not respond to added vitamin B_1 either

TABLE IV

EFFECT OF PHOTOPERIOD AND VITAMIN B_1 ON THE GROWTH OF COSMOS GROWN IN SAND CULTURE. PLANTS HARVESTED FOUR WEEKS AFTER APPEARANCE OF FIRST LEAF

	SHORT PHOTOPERIOD				LONG PHOTOPERIOD			
	DRY WEIGHT PER PLANT		NUMBER OF PLANTS	VITAMIN B_1 PER SHOOT	DRY WEIGHT PER PLANT		NUMBER OF PLANTS	VITAMIN B_1 PER SHOOT
	TOPS	ROOTS			TOPS	ROOTS		
	mg.	*mg.*		γ	*mg.*	*mg.*		γ
Control	53	9.2	90	0.26	74.5	15.0	120	0.38
Added vitamin B_1	67	18.0	60	0.42	135.0	26.0	90	0.86

under long photoperiod or short photoperiod, although growth is decreased under the latter condition (table V).

TABLE V

EFFECT OF PHOTOPERIOD AND VITAMIN B_1 ON THE GROWTH OF TOMATO PLANTS IN SAND CULTURE. PLANTS HARVESTED THREE WEEKS AFTER APPEARANCE OF SECOND LEAF

	SHORT PHOTOPERIOD				LONG PHOTOPERIOD			
	DRY WEIGHT PER PLANT		NUMBER OF PLANTS	VITAMIN B_1 PER SHOOT	DRY WEIGHT PER PLANT		NUMBER OF PLANTS	VITAMIN B_1 PER SHOOT
	TOPS	ROOTS			TOPS	ROOTS		
	mg.	*mg.*		γ	*mg.*	*mg.*		γ
Control	540	70	10	2.86	1050	184	10	6.78
Added vitamin B_1	540	70	10	3.18	1260	248	10	5.67

Discussion

In an earlier paper (3) it has been shown that the leaves of the different species of plants vary greatly in their content of vitamin B_1. Different

species presumably vary then in the amount of vitamin B_1 which they are able to synthesize. It has also been shown that of these various species, those which synthesize relatively large amounts of vitamin B_1 do not respond to the addition of this substance with increased growth, while those species which produce relatively little vitamin B_1 do respond to its addition with increased growth. The foregoing data show that with both Xanthium and Brassica, the amount of vitamin B_1 present in the plant is influenced by the photoperiod to which the plant is subjected. This is not unexpected in view of the fact that vitamin B_1 is formed by higher plants in the presence of light (2). The data also indicate that with Xanthium and Brassica the growth response to added vitamin is greater under conditions of short photoperiod (low vitamin B_1 synthesis) than it is under conditions of long photoperiod (higher vitamin B_1 synthesis). In the present experiments the vitamin B_1 content of the plant varies with the photoperiod; in the earlier experiments the vitamin B_1 content of the plant varied with the species but in both cases, relatively low vitamin B_1 content may be correlated with relatively large growth response to added vitamin B_1.

Summary

1. Plants of Xanthium, *Brassica alba, Brassica nigra,* Cosmos, and Lycopersicum all contain more vitamin B_1 when grown under conditions of long photoperiod (18 hours) than when grown under conditions of short photoperiod (9 hours).

2. Xanthium and Brassica respond to additions of vitamin B_1 more strikingly under the conditions of short photoperiod than under those of long photoperiod.

3. It is suggested that the effect of photoperiod on the vegetative growth of plants may be mediated by the effect of photoperiod upon the production, not only of vitamin B_1, but also on the production of other growth factors.

WILLIAM G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES
CALIFORNA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

LITERATURE CITED

1. BONNER, J. Vitamin B_1 a growth factor for higher plants. Science n.s. **85**: 183–184. 1937.
2. ————, and GREENE, J. Vitamin B_1 and the growth of green plants. Bot. Gaz. **100**: 226–237. 1938.
3. ————, ————. Further experiments on the relation of vitamin B_1 to the growth of green plants. Bot. Gaz. **101**: 491–500. 1939.
4. ČAJLACHJAN, M. CH., and ŽDANOVA, L. P. Hormones of growth in for-

mation processes. I. Photoperiodism and creation of growth hormones. Compt. Rend. Acad. Sci. U.S.S.R. **19**: 107–111. 1938.
5. CRIST, J., and STOUT, J. Relation between top and root size in herbaceous plants. Plant Physiol. **4**: 63–85. 1929.
6. GARNER, W. "Photoperiodism," in Biological Effects of Radiation, vol. II. 677–713, New York, 1936.
7. ———, and ALLARD, H. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**: 553–606. 1920.
8. HAMNER, K. C., and BONNER, J. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100**: 388–431. 1938.
9. MOSKOV, B. S. Photoperiodism and a hypothesis as to hormones of flowering. Compt. Rend. Acad. Sci. U.S.S.R. **15**: 211–214. 1937.
10. MURNEEK, A. E. Biochemical studies of photoperiodism in plants. Univ. Missouri Agr. Exp. Sta. Res. Bull. 268. 1937.
11. TAGEEVA, SOPHIE. Zur Frage des Zusammenhangs zwischen Assimilation und Ertragsfähigkeit. Planta **17**: 758–793. 1932.
12. WEAVER, J., and HIMMEL, W. Relation between development of root system and shoot under long and short day illumination. Plant Physiol. **4**: 435–457. 1929.
13. WITHROW, R. B., and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wave length of artificial light used to lengthen the daylight period. Plant Physiol. **11**: 225–249. 1936.

OBSERVATIONS ON THE DEVELOPMENT OF CERTAIN CELL-WALL CONSTITUENTS OF FORAGE PLANTS[1]

EMMETT BENNETT

(WITH TWO FIGURES)

Introduction

In the conventional methods of "proximate analysis," pectic substances, hemicelluloses, and lignin, for the most part, appear in the "nitrogen-free extract" fraction. It is now generally recognized that this method of differentiation gives very little information as to the nature of the substances composing these fractions; hence attempts are being made to ascertain their chemical constitution, nutritional value, precursors, and relationships in the plant. In this way a better understanding of the metabolism and economic value of plants will be obtained.

The changes which the cell-wall constituents undergo during the development of the plant are not definitely known. True chemical relationships cannot be established as yet because the chemical constitution of lignin is not known. Transformation studies have been made, however, and a number of theories have been evolved. The principal theories as summarized by NORMAN (14) are:

1. That pectin undergoes transformation to lignin.
2. That the hemicelluloses or certain groups thereof may be converted to lignin.
3. That the polyuronide hemicelluloses are formed from pectin.
4. That all three substances are connected, lignin being formed from pectin through the intermediate stage of polyuronide hemicelluloses.

Most investigations of this kind have dealt with woody tissues, while a few have dealt with grasses, especially cereal grasses. Instances are very few, however, in which transformation studies of these compounds have been made during the growing period of any one species.

This paper presents results of a 2-year study of the content of pectin, hemicelluloses, and lignin in Kentucky blue grass (*Poa pratensis*) and red clover (*Trifolium pratense*) taken at successive stages of growth, with special reference to the formation of lignin.

Review of literature

The literature is both obscure and contradictory. No attempt is made to give a complete review; instead, the reader is directed to the relatively recent publications by PHILLIPS (16) and NORMAN (14).

[1] Published as Contribution no. 343 of the Massachusetts Agricultural Experiment Station.

In general, transformation theories assume that pectic substances are eventually replaced by lignin. The intermediate steps, however, are problematical. FUCHS (10) assumes that the primary reduction products of the cell-wall constituents, especially pectin, are transformed into phenols, which by further changes, especially condensations, may result in the formation of lignin. EHRLICH (9) also considers pectin to be an intermediate product in this synthesis. He assumes, however, that the transformation comes about through enzymatic action involving decarboxylations, dehydrations, and reductions. Other investigators obtained analytical data which indicated that some such change occurs. CANDLIN and SCHRYVER (7) note that lignified tissues contain relatively large amounts of lignin and hemicelluloses, with only traces of pectin; non-lignified tissues, on the other hand, contain relatively large amounts of pectins, small amounts of hemicelluloses, and no lignin. MEHTA (11) and RITTER (19), using pectic solvents, fail to find any pectin in the middle lamella of mature woody tissue. O'DWYER (15) suggests that the transformation takes place through the hemicelluloses. This suggestion is supported by BAILEY's (3) work on the middle lamella of Douglas fir in which he found lignin associated with pentosans.

Other investigations have disclosed no definite support for the above transformation theories. ANDERSON (1) concludes from his work on black locust wood that pectic substances are laid down early in growth and remain unchanged as the wood ages. A study of the pectic substances in the developing pods of beans and sweet peas by BUSTON (6) reveals no direct evidence for the transformation of pectin to lignin. A new and different way of explaining the whole problem is offered by BAILEY (4) in which he states that because of the difference in cell structure it is not essential to account for the various ratios of these substances in different plants on the basis of transformation.

Materials and methods of analysis

Samples of Kentucky blue grass were obtained from the Massachusetts State College Farm at Amherst, Massachusetts, for the years 1935, 1936, and 1937, and of red clover for the years 1936, and 1938. The first clippings of the grass were taken when about three inches tall; subsequent clippings were made every 5 to 8 days through the blooming stage. Clover clippings were not obtained as early in the season but were made in the same way as the grass thereafter. All samples were dried in a forced draft oven at 60–65° C.

The following methods of analysis were used on finely ground samples.

Ash, nitrogen, and *total furfural* were determined by the A.O.A.C. (2) methods.

Total pectin.—All samples were extracted with alcohol prior to an exhaustive extraction with 0.6 per cent. ammonium citrate (8).

Total hemicelluloses.—The procedure outlined by BUSTON (5) was adopted.

Lignin.—A modified SCHWALBE (20) method was used on material which had first been extracted with an alcohol-benzene solution.

Experimentation

All results obtained are presented in tabular form; those for pectin, hemicelluloses, and lignin are also shown graphically.

TABLE I

PARTIAL PERCENTAGE COMPOSITION OF KENTUCKY BLUE GRASS AT DIFFERENT STAGES OF GROWTH FOR THE YEARS 1935, 1936, AND 1937 ON A DRY MATTER BASIS

DATE OF SAMPLING	ASH	NITROGEN	TOTAL PECTIN	TOTAL HEMICELLULOSES	LIGNIN	TOTAL FURFURAL
	%	%	%	%	%	%
4/26/35	7.05	4.06	0.67	14.24	2.86	6.25
5/ 1/35	5.93	3.35	0.78	19.12	3.53	7.56
5/ 9/35	5.91	2.70	0.86	22.58	5.06	8.86
5/16/35	4.82	2.03	0.74	26.44	6.33	10.48
5/21/35	5.10	1.87	0.90	26.00	6.88	10.20
5/27/35	4.25	1.50	0.96	27.76	7.75	11.97
5/ 8/36	7.30	3.65	0.90	18.07	4.50	8.72
5/13/36	7.00	3.39	0.93	19.51	5.86	8.48
5/20/36	6.58	2.66	0.95	20.22	6.83	9.78
5/25/36	5.80	2.20	0.59	20.47	7.62	11.16
6/ 1/36	4.96	1.66	0.62	21.55	8.50	11.20
6/ 5/36	4.85	1.57	0.52	21.01	8.45	11.77
6/10/36	4.46	1.21	1.11	25.08	10.65	12.56
5/16/37	7.31	3.34	0.87	20.41	3.17	9.59
5/21/37	7.47	2.92	0.85	21.36	3.79	9.79
5/26/37	6.96	2.54	0.74	22.15	4.78	10.78
6/ 2/37	6.56	2.21	0.78	24.73	6.40	11.70
6/ 9/37	6.17	1.78	0.83	25.23	6.83	11.72

TOTAL ASH

In general, the percentage content of ash in both plants decreased as the season progressed. The percentage of ash in the early part of the season was consistently greater in red clover. Both these observations are in agreement with similar work of others.

TOTAL NITROGEN

The percentage of total nitrogen in both plants followed practically the same seasonal trend as that of the ash. Red clover contained a slightly greater percentage of nitrogen than did Kentucky blue grass.

TABLE II

PARTIAL PERCENTAGE COMPOSITION OF RED CLOVER AT DIFFERENT STAGES OF GROWTH FOR THE YEARS 1936 AND 1938 ON A DRY MATTER BASIS

DATE OF SAMPLING	TOTAL ASH	TOTAL NITROGEN	TOTAL PECTIN	TOTAL HEMICELLULOSES	LIGNIN	TOTAL FURFURAL
	%	%	%	%	%	%
5/ 8/36	10.22	4.80	5.61	6.93	4.82	4.85
5/13/36	9.23	4.11	5.62	8.02	5.11	5.12
5/20/36	9.09	3.71	5.02	8.45	5.40	5.58
5/25/36	5.77	3.01	5.22	9.00	6.18	6.12
6/ 1/36	4.94	2.97	4.93	9.86	6.83	5.50
6/ 5/36	4.62	2.63	5.13	11.19	9.20	6.51
6/10/36	4.31	2.69	5.53	11.38	9.48	6.58
5/14/38	10.85	3.63	7.19	4.30		5.20
5/19/38	10.37	3.53	8.39	5.69	3.71	5.58
5/25/38	9.93	3.07	8.15	5.68	3.87	6.25
6/ 1/38	9.92	2.84	8.07	6.98	4.80	6.35
6/ 9/38	8.92	2.75	7.89	7.00	5.82	6.61
6/20/38	7.72	2.43	6.99	8.48	8.32	6.81

TOTAL PECTIN

Pectic substances in Kentucky blue grass were present in small and relatively constant amounts. BUSTON (5) working with forage grasses found that they are definitely low in pectin and that the relative amounts of hemicelluloses and pectin are similar to those in wood and straw.

Red clover on the other hand contained relatively large amounts, which showed no definite seasonal trend. The results of the last year, however, showed a slight but insignificant decrease as the plant grew older. A comparison of the pectic content of this plant with that of certain structures

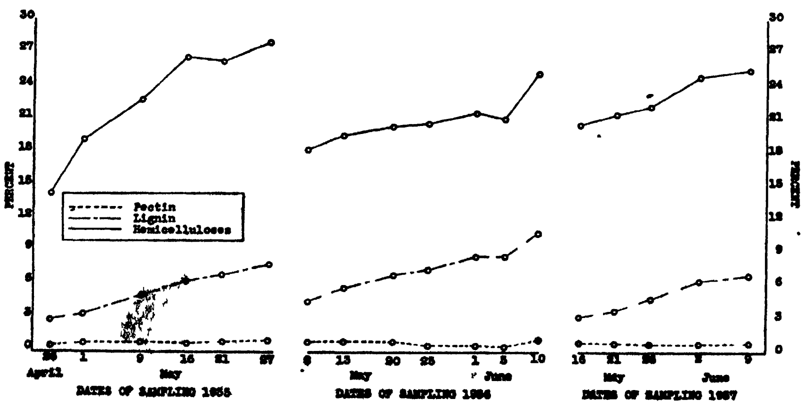


FIG. 1. Percentages of pectin, hemicelluloses, and lignin in Kentucky blue grass at successive stages of growth.

from other plants (6, 12) revealed that the amounts present, in 1938 especially, were similar to leaves.

An inspection of figures 1 and 2 will disclose the relative differences in

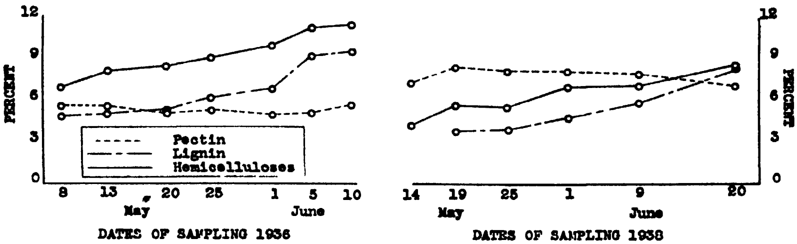


FIG. 2. Percentages of pectin, hemicelluloses, and lignin in red clover at successive stages of growth.

content of pectin of the two plants, and the fact that the percentage content of the last clipping for either plant in every case was practically equal to or greater than that in the first stage of growth. These results do not suggest that a transformation of pectin has taken place. BUSTON (6) working with developing pods of beans and sweet peas, obtained results of a similar nature.

HEMICELLULOSES

With a few minor exceptions the percentage of hemicelluloses in both plants increased as the season advanced. Relatively high percentages of this group were found in both species at a very early stage. The percentage in red clover was somewhat less than half that in Kentucky blue grass and was, in some cases, associated with a similar percentage of pectin. In mature Kentucky blue grass the ratio of hemicelluloses to pectin was about twenty-five to one. These two examples clearly illustrate the ratio of hemicelluloses to pectin that is generally expected in non-lignified and lignified tissue respectively.

TOTAL FURFURAL

No attempt was made to account for the furfural on the basis of source material. The percentage found, therefore, represents furfural contributed by pentosans, pentoses, and uronic acids, which for the most part came from the hemicelluloses and pectins. In general the percentage of total furfural increased in both species as the season advanced.

LIGNIN

Lignin, like the hemicelluloses, was present in both species in the earliest stage of growth and increased in content as the season progressed. The maximum values differed but very little between the species. This fact is unusual in view of the large species differences in the percentages of pectin,

and hemicelluloses. Seasonal trends are in general agreement with the reports of others (**13**, **17**, **18**).

Discussion

The problem of expressing data obtained from a series of samples representing different ages and sizes, with the hope of illustrating the actual metabolic picture, is difficult. The successful expression of data of this kind on a percentage basis may be masked because other constituents vary in percentage content from time to time; the expression then is one of relative concentration and does not account for the actual quantities involved during growth. A decreasing percentage of a constituent may actually represent an increase in the absolute quantity elaborated. On the other hand, for constituents present in small percentages which remain constant or increase, there can be but little doubt that the quantities increase during the growing process.

In general, the theories involving the formation of lignin are based upon the assumption that hemicelluloses and pectins are labile; hence it is assumed that pectin will eventually disappear. The figures obtained in this investigation show that the pectin content remains relatively constant while other constituents are on the increase. This indicates that pectin is at least formed faster than it is transformed. This indication, together with the fact that red clover contains an amount of pectin in some cases equal to the content of lignin, which in turn is similar in amount to that found in plants having only traces of pectin, is contrary to the above theory.

If hemicelluloses are to be considered precursors of lignin it is quite evident that the percentage of lignin in Kentucky blue grass should have been much higher, or that of red clover much lower. In considering the hemicelluloses as intermediate compounds between pectin and lignin, it will be noticed that the amounts of these substances bear the opposite rather than the expected quantitative relationship to each other.

It is interesting to note that hemicelluloses and lignin were found in definite amounts in the very earliest samples obtained. These observations suggest that the compounds are likely to be active metabolic products rather than products characteristic of maturity in plants.

From the chemical data, no direct relationship between the three substances is apparent. In the absence of cytological data it would seem that the evidence favors BAILEY's (**4**) explanation; namely, that the various ratios of these substances may be accounted for on the basis of cell structure.

Summary

A study was made of the ash, nitrogen, pectin, hemicelluloses, and lignin in Kentucky blue grass and red clover at successive stages of growth, with special reference to the formation of lignin.

In general the percentages of ash and total nitrogen decreased throughout the growing season.

The percentages of pectin in both species remained relatively constant during the growth periods. Kentucky blue grass was similar to wood or straw in percentage of pectin, while red clover was comparable to leaves.

The percentage of hemicelluloses increased in both species as the season progressed. The percentage of hemicelluloses in mature Kentucky blue grass was about twenty-five times greater than that of pectin, while in red clover the content of hemicelluloses was sometimes less than that of pectin and never more than about twice as large.

The percentage of lignin in both species increased as the plants grew older. The maximum percentage was about the same for both species.

Approximately the same percentage of lignin was associated with different proportions of pectin and hemicelluloses in the two species.

Lignin and hemicelluloses were found at a very early age.

No direct evidence was obtained for a relationship between pectin, hemicelluloses, and lignin. The results suggest rather that the different proportions of these substances may be attributed to a difference in cell structure.

Massachusetts Agricultural Experiment Station,
Amherst, Massachusetts

LITERATURE CITED

1. Anderson, E., Seigle, L. W., Krznarich, P. W., Richards, L., and Marteny, W. W. The isolation of pectic substances from wood. II. Jour. Biol. Chem. **121**: 165–174. 1937.
2. Assoc. Offic. Agr. Chem. Official and tentative methods of analysis. Compiled by the Committee on editing methods of analysis. Third edition. Washington, D. C. 1930.
3. Bailey, A. J. Lignin in Douglas fir. Ind. & Eng. Chem. Anal. Ed. **8**: 389–391. 1936.
4. Bailey, I. W. Cell wall structure of higher plants. Ind. & Eng. Chem. **30**: 40–47. 1938.
5. Buston, H. W. The polyuronide constituents of forage grasses. Biochem. Jour. **28**: 1028–1037. 1934.
6. ————. Observations on the nature, distribution and development of certain cell-wall constituents of plants. Biochem. Jour. **29**: 196–218. 1935.
7. Candlin, E. J., and Schryver, S. B. Investigations of the cell-wall substances of plants, with special reference to the chemical changes taking place during lignification. Proc. Roy. Soc. (London) B **103**: 365–376. 1928.

8. CONRAD, C. M. A biochemical study of the insoluble pectic substances in vegetables. Amer. Jour. Bot. **13**: 531–547. 1926.

9. EHRLICH, F. Über die Chemie des Pektins und seine Beziehungen zur Bildung der Inkrusten der Cellulose. Cellulosechemie **11**: 161–170. 1930. Cited by (**18**).

10. FUCHS, W. M. The chemical nature of lignin. Paper Trade Jour. **102**: 33–36. March 26, 1936.

11. MEHTA, M. M. Biochemical and histological studies on lignification. Part II. Histological studies on the polysaccharides and aromatic constituents of the cell wall. Biochem. Jour. **19**: 979–997. 1925.

12. NANJI, D. R., and NORMAN, A. G. The estimation of the individual pectic substances in nature. Biochem. Jour. **22**: 596–604. 1928.

13. NORMAN, A. G. A preliminary investigation of the development of structural constituents in the barley plant. Jour. Agr. Sci. **23**: 216–227. 1933.

14. ——————. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford U. Press, London, 1937.

15. O'DWYER, M. H. Preliminary investigations on the constitution of the hemicelluloses of timber. Biochem. Jour. **22**: 381–390. 1928.

16. PHILLIPS, M. The chemistry of lignin. Chem. Rev. **14**: 103–170. 1934.

17. ——————, DAVIDSON, J., and WEIHE, H. D. Studies of lignin in wheat straw with reference to lodging. Jour. Agr. Res. **43**: 619–626. 1931.

18. ——————, and GOSS, M. J. Composition of the leaves and stalks of barley at successive stages of growth, with special reference to the formation of lignin. Jour. Agr. Res. **51**: 301–319. 1935.

19. RITTER, G. L. Distribution of lignin in wood. Ind. & Eng. Chem. **17**: 1194–1197. 1925.

20. SCHWALBE, H. Über neue Methode zur Bestimmung des Lignins. Papierfabr. **23**: 174–177. 1925.

SOIL TEMPERATURE AND GROWTH OF MARQUIS WHEAT

D. J. WORT

(WITH THREE FIGURES)

Introduction

The development of plants as influenced by soil temperature, as distinct from air temperature, has not been investigated as fully as have growth responses to other environmental factors.

Broadly speaking, roots grow best at lower temperatures than the shoots and they suffer considerably at higher temperatures. BRENCHLEY (2) records injury and death of plants from high root temperatures in water cultures. BUSHNELL (3) grew potatoes at different soil temperatures and obtained the highest yield of tubers at 18° C., but maximum top growth occurred at 21° C.

From the time of SACHS's classical experiment in 1860 it has been known that decreased soil temperature results in decreased absorption of water by plants. SACHS (8) placed a well-watered tobacco plant in a warm room and surrounded the pot with ice. After a brief period the plant began to wilt, but when the ice was removed and the soil heated it recovered without the addition of water. KRAMER (7), using a porcelain absorbing surface, determined that increasing soil temperatures (0° C. to 43° C.) materially increased the movement of water from the soil, or in other words, increased the water supplying capacity of the soil.

Transpiration of *Helianthus annuus* varies very little with soil temperatures between 55° F. and 100° F., drops rapidly below 55° F., is reduced to half at 38° F., and approaches zero at 32° F., according to the findings of CLEMENTS and MARTIN (4).

JONES (5) reports that low soil temperatures are responsible for chlorosis of gardenias. His four conclusions are: 1. color of leaf is affected by soil temperature; 2. size of leaf and rate of growth are related to soil temperature and not to air temperature, leaf size increases with increasing temperatures up to 32° C.; 3. bud set is best obtained by growing plants at high soil temperature and then dropping the temperature to a point where vegetative development is stopped; 4, air temperature is of little importance, and that only in as much as it affects soil temperature.

Soil temperature effects on blooming were investigated by ALLEN (1). He found that the flowering of some varieties of stocks was affected but little by soil temperature; higher temperatures increased the length of time to bloom and the number of flowers per plant of snapdragons. The morphology of wheat seedlings as related to soil temperature was studied by TAYLOR and MCCALL (9).

Jones *et al.* (6) state that the wheat plant functions best in all stages of its development at relatively low soil temperatures. The favoring influence of low temperature is more marked during the seedling stage. While the wheat (Marquis and Turkey) started germination most promptly at 24° to 28° C., germination was more uniform and stronger plants resulted at low soil temperatures, of about 8° to 10° C. Although the higher soil temperatures stimulated the early development of tops, these too were ultimately stronger at the lower temperatures. The best plant, as measured by stockiness and early maturity, developed at a soil temperature of about 16° C.

Experimentation

An experiment to determine the response of Marquis spring wheat to various soil temperatures was conducted during the summer of 1937 and repeated with slight modifications and more numerous temperatures during the summers of 1938 and 1939.

Method

Marquis wheat was planted one inch deep in good loam soil in two-gallon porcelain containers. The containers were submerged in water in covered tanks to within 2 inches of their tops. The temperature of the water was thermostatically controlled with the exception of the 22° C. tank. Twenty-two degrees was the lowest temperature that could be obtained by using continuously running water from the water mains and the temperature of the water (hence of the soil in the container) in this tank rose at times to 24° C. and fell to 21° C. Fluctuations of 1° C. in the other tanks were seldom. The dates of planting, harvest, durations of the experiments, number of replicate containers at each soil temperature, number of plants and the soil temperatures used are given in table I.

TABLE I

Dates of planting, harvest, durations of experiments, and soil temperatures used

Year	Date of planting	Date of harvest	Pots at each temperature	Total plants at each temperature	Duration of experiment	Soil temperatures °C.
1937	July 1	Aug. 27	2	10	57 days	22°, 33°, 44°
1938	July 9	Aug. 26	2	10	48 "	22° to 44° in intervals of 2 degrees
1939	July 3	Sept. 11	5	15	70 "	22° to 42° in intervals of 4 degrees

In 1937 and 1939 the wheat was allowed to grow at a soil temperature of 26° C. for 5 days before the final soil temperatures, given in table I, were established and maintained. In this way the plants later grown at the various soil temperatures were well established. The 1938 temperatures were maintained from the beginning, the grain being sown in soils at these temperatures. The grain failed to germinate at 42° and 44° C., and although resown, the wheat failed to grow. In the 1937 experiment all plants grown at 44° C. died within 35 days.

The temperature in the greenhouse was uncontrolled and rose nearly every day to at least 100° F., falling at night to approximately 70° F.

The pots were given sufficient water once a day to keep the soil at all temperatures moistened to approximately the same degree. More water had to be added to the higher temperature pots than to the lower. Drains from the containers provided constant drainage of excess water.

When the plants were harvested the soil was washed from the roots and the roots then rinsed several times in tap water which removed practically all adhering soil particles. The roots were then rinsed several times in a 5 per cent. sodium chloride solution and finally in distilled water.

Results and discussion

As mentioned above the wheat failed to grow at soil temperatures of 42° and 44° C. in the 1938 experiment. These temperatures were maintained from the time of sowing. In the 1937 experiment all plants grown at 44° C. died within 35 days.

The data for the three experiments are very similar and those for 1939, being representative, are the only ones tabulated. Significant differences are mentioned in the discussion.

TABLE II

GROWTH AND HARVEST DATA, 1939

Soil temperature	Average height at 14 days	Average height at 70 days	Average greatest root length at 70 days	Average tillers per plant at 70 days	Average days to head	Average dry weight per plant 70 days			Top/Root ratio
						Tops	Roots	Total	
°C.	in.	in.	in.		days	gm.	gm.	gm.	
22	10.1	25.0	12.2	4.3	65	1.61	0.40	2.01	4.0
26	10.2	22.5	12.1	3.5	63	0.96	0.23	1.19	4.2
30	9.3	20.1	9.9	2.3	59	0.62	0.11	0.73	5.6
34	8.4	16.4	4.4	1.6	54	0.21	0.03	0.24	7.0
38	7.0	11.5	3.3	1.3	61	0.14	0.03	0.17	4.7
42	6.0	7.2*	2.3*	1.4*		0.06*	0.013	0.073	4.6

* Only 8 of the original 15 plants survived at 70 days.

Height of plants, root length, and tiller number

The heights of the plants, measured from the soil level to the tip of the longest leaf of the leading tiller, were taken throughout the growing period at weekly intervals.

The curves of the average growth of the plants at each soil temperature are shown in figure 1. The comparative heights of the plants at 14, 21, 28,

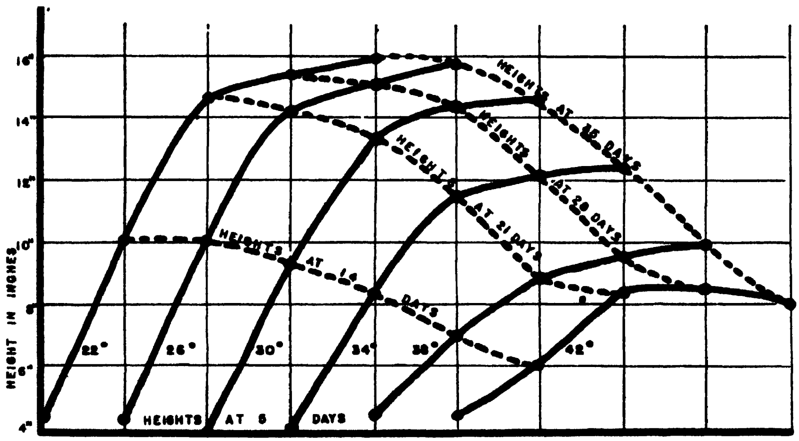


Fig. 1. Curves of the average growth of Marquis wheat from 5 days to 35 days. The solid graphs represent the average growth of plants at the soil temperatures indicated. The broken lines are the height curves at the ages stated.

and 35 days are given by the broken line. The decrease in height of the plants grown at a soil temperature of 42° C. between the age of 28 and 35 days is attributed to considerable death of leaves in this interval. The height of these plants at 70 days was 7.2 inches, whereas the average height at the age of 28 days was 7.47 inches.

Growth of plants at soil temperature of 26° C. for the first 5 days, and then at various soil temperatures (as in 1937 and 1939), produced greater height at the higher temperatures and lesser height at lower temperatures, measured 14 days after planting, compared with plants grown at the various soil temperatures from the start (as in 1938). This treatment also enabled the plants to withstand high soil temperatures for an additional 65 days. The heights at the same temperatures 42 days after planting were very similar in the three experiments, differing by less than an inch in most cases. The differences caused by the initial treatment had been overcome.

The number of tillers decreased from 4.3 at 22° C. to 1.4 at a soil temperature of 42° C., as shown in table II. A corresponding decrease in yield of grain would be expected to follow owing to the reduction in the number of heads.

The length of roots decreased with increasing temperatures, the greatest

decrease occurring between 30° and 34° C. The greatest decrease in stem height occurred between 34° and 38° C. The greater decrease in roots than in stems, relatively, between 30° and 34° C. results in an increased top root ratio at these temperatures, but the later decrease in tops restores the ratio to approximately the same value as at the lower soil temperatures used.

Weights of tops and roots and top root ratio

Dry weights of tops and roots and total dry weight decreased with increasing temperatures as shown in table II. These values are expressed graphically in figure 2.

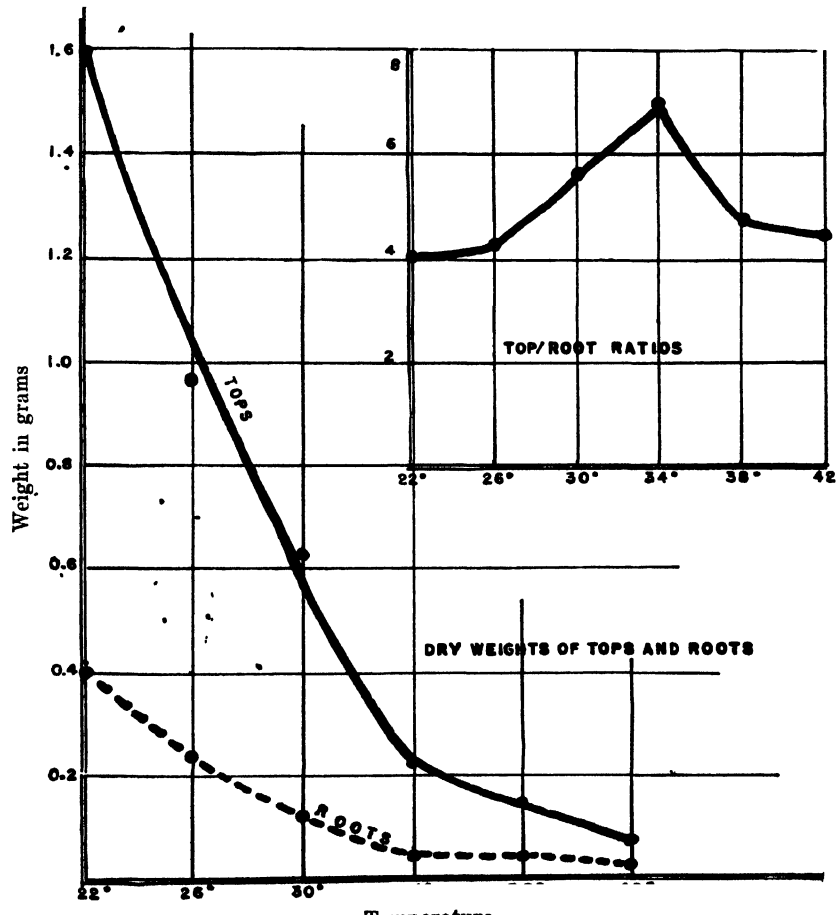


Fig. 2. Dry weights of tops and roots and the top root ratios. The weights are the average per plant, expressed in grams, for plants grown at the soil temperatures indicated.

In the 1939 experiment the top root ratio rose from 4.0 at 22° to 7.0 at 34° C. and fell thereafter to 4.6 at 42° C. This is indicative of a greater comparative reduction in roots than in tops as the soil temperature rises to 34° C. Above this temperature the falling ratio indicates a greater reduction in tops in comparison with roots. The results of the former experiments indicate the same ratio behavior. In 1938 the ratio rose from 4.6 at 22° to 7.1 at 30° C., falling once more to 3.0 at 40° C. It is apparent, therefore, that with increasing soil temperatures the top root ratio increases reaching a maximum at 30°–34° C., and falls again at higher soil temperatures. The findings of these experiments indicate that the general statement found in the literature that roots suffer more than shoots at higher temperatures is true for Marquis wheat only to 30°–34° C. Above these temperatures the reverse holds true.

Size and color of leaves

Observations as to the leaf size and color were made when the plants were 42 days old. Leaf length, width, and area were at a maximum at 22° C., the lowest temperature used in the experiments. The leaves became visibly lighter in color as the soil temperature increased above 32° C.

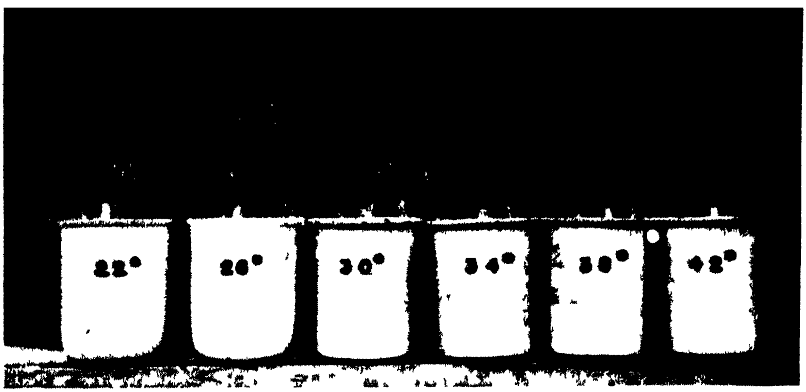


Fig. 3. Plants of the 1939 experiment when harvested at 70 days. The plants are representative of 15 grown at soil temperatures shown on the pots, expressed in degrees centigrade.

Heading

Heading was assumed to have occurred when the head had completely emerged from the sheath. The Marquis wheat grown in 1937 at a soil temperature of 22° C. headed in an average of 57 days, while that grown at 33° C. headed in an average of 51 days. The higher soil temperature in this

case accelerated heading by 6 days. No plants headed within 48 days, the duration of the 1938 experiment.

The 1939 heading data are included in table II. The average heading time was 65 days at 22° and 54 days at 34° C., a decrease of 11 days. At 38° C. the average time to head increased to 61 days. A number of heads were sterile at this temperature. No heads were formed at 42° C. Heading was thus accelerated as the soil temperature rose from 22° to 34° C., but was retarded or prevented at higher temperatures.

Summary

1. Marquis spring wheat was grown in the greenhouse at soil temperatures ranging from 22° to 44° C. over a period of three years.

2. Plant height, root length and extent, and tiller number decreased as the soil temperatures rose from 22° to 42° C.

3. Greatest dry weights of tops and roots and total dry weight, at time of harvest, resulted in plants grown at a soil temperature of 22° C., the lowest maintained in these experiments. The top root ratio reached a maximum at temperatures of 30° to 34° C., falling again as the soil temperature was further increased.

4. Leaves were largest at 22° C. and became lighter in color as the soil temperature rose above 32° C.

5. Increasing the soil temperatures from 22° to 34° C. accelerated heading by as much as 11 days. Soil temperatures above 34° C. retarded or prevented earing.

The University of Chicago

LITERATURE CITED

1. Allen, R. C. The effect of soil temperature on the growth and flowering of certain greenhouse crops. Proc. Amer. Soc. Hort. Sci. **32**: 635–637. 1934.
2. Benchley, W. E. On the relations between growth and the environmental conditions of temperature and bright sunshine. Ann. Appl. Biol. **6**: 211–244. 1920.
3. Bushnell, J. The relation of temperature to growth and respiration in the potato plant. Minnesota Agr. Exp. Sta. Tech. Bull. 34. 1925.
4. Clements, F. E., and Martin, E. V. Effect of soil temperature on transpiration in *Helianthus annuus*. Plant Physiol. **9**: 619–630. 1934.
5. Jones L. H. Is soil temperature important? Florists Exch. and Hort. Trade World. Jan. 15, 1938.
6. ————, Johnson, J., and Dickson, J. G. Wisconsin studies upon the relation of soil temperature to plant disease. Wisconsin Agr. Exp. Sta. Res. Bull. 71. 1926.

7. Kramer, P. J. Effects of soil temperature on the absorption of water by plants. Science n. s. **79**: 371–372. 1934.
8. Sachs, J. Beiträge zur Lehre von der Transpiration der Gewächse. Bot. Zeit. **18**: 121–126. 1860.
9. Taylor, J. W., and McCall, M. A. Influence of temperature and other factors on the morphology of the wheat seedling. Jour. Agr. Res. **52**: 557–568. 1936.
10. Wort, D. J. Vernalization of Marquis wheat and other spring cereals. Bot. Gaz **101**: 457–481. 1939.

EFFECT OF CERTAIN THIOCYANATE SPRAYS ON FOLIAGE AND FRUIT IN APPLES[1]

R. B. DUSTMAN AND I. J. DUNCAN

(WITH ONE COLORED PLATE)

As set forth briefly in a recent publication (5) the authors have found that certain thiocyanates, applied as sprays to the foliage and fruit during the growing season, affect the formation of red color in apples. This red pigment has been shown (4, 6) to be idaein, a glycoside which yields cyanidin and galactose on hydrolysis. Growers in general recognize the importance of good color in apples, and any practical procedure which improves color may offer an advantage from the standpoint of sale value.

During the summer of 1936 amid general trials with numerous substances including both organic and inorganic materials, some of which were applied as injections, others as sprays, and still others as dips or immersion treatments, observations suggested that color was being affected in those fruits receiving sprays of soluble thiocyanates. Further trials during the following season confirmed the earlier results and indicated that the thiocyanate ion was the effective agent, irrespective of the positive ion with which it was associated. DENNY (1, 2, 3) has already shown that sodium, potassium, and ammonium thiocyanates exhibit an unusual physiological effect on dormant potato tubers by breaking or shortening the normal rest period.

Materials and methods

EQUIPMENT AND CONTROLS

During the summers of 1937 and 1938 all materials used were applied to individual limbs with a small hand-sprayer while nearby limbs were protected from drifting spray by sheets of heavy paperboard. In 1939 most of the applications were made with power-spray outfits. In the power-spray trials entire trees were sprayed except for one limb of each tree, which was selected as a control and covered with a heavy canvas during the spraying operation.

VARIETIES

The varieties tried thus far have included Duchess, Red Duchess, Melba, McIntosh, Maiden Blush, Wagner, Delicious, Jonathan, Stayman, Rome, York, Wealthy, Winesap, Spy, Baldwin, Grimes, Golden Delicious, Yellow Transparent, Early Harvest, and several others.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 229.

Thiocyanates

In the hand-spray trials the substances used have included combinations of the thiocyanate radical with sodium, potassium, calcium, magnesium, barium, copper, lead, ammonium, methyl, ethylene, isopropyl, n-butyl, urea, hydrazine, aniline, pyridine, trimethyl amine, triethanol amine, nicotine, and other groups. In these trials certain of the thiocyanates were tried in mixtures with other common spray materials such as arsenate of lead, bordeaux, lime sulphur, and flotation sulphur.

In the power-spray trials sodium thiocyanate was the only thiocyanate used, and it was applied alone in all instances.

Concentrations

The concentrations employed in the hand work have varied from 0.05 per cent. to 0.5 per cent. (in the case of certain insoluble substances suspensions of corresponding strength were used, sometimes with the addition of other materials to increase the solubility of the desired substance). The concentrations of sodium thiocyanate in the power-spray trials varied from 0.05 per cent. to 0.2 per cent.

Time and number of applications

The number of sprays applied has varied from one to five, and the time of application has varied from June to September, inclusive, depending upon the variety under test, its stage of maturity, and the character and degree of response obtained. When more than one spray was applied, applications were usually made at intervals of approximately ten days. With late fall and winter varieties two to four sprays applied during August and early September in general gave best results.

Results

Effect on foliage

Virtually all of the thiocyanate sprays that have produced color effects have resulted also in a greater or lesser amount of injury to the foliage. In some instances where the materials were applied in too great concentration, or too frequently, this injury has been excessive; in other instances the amount of leaf-burn has been little or no more than would be expected from other spray materials in common use. The first noticeable effect of the spray is a marginal burn of varying width at the edges and tips of the leaves which makes itself apparent over a period of several days following the application of the thiocyanate. Later, in many leaves, particularly following additional applications, a chlorotic area or band develops inside the marginal burn, which has now become brown in appearance. Some of the

smaller and more tender leaves of the leaf clusters may turn yellow and fall. Repeated applications of spray result in an increase of the chlorotic tissue, but the portions of the leaf next the midrib remain green. Many of the leaves tend to curl slightly at the edges where the margins are brown.

An interesting feature of this chlorotic condition is its tendency to disappear as the sprays are discontinued and the season advances. Thus there is a partial recovery of the tree from the shock sustained, which in some instances has been so complete that the only abnormal appearance at the end of the season was a narrow brown strip at the margins of the injured leaves. Sprayed and unsprayed foliage was apparently almost equally green.

Another feature of interest is the observation that the foliage of the sprayed limbs or trees appears to be retained equally well in the fall with that of the unsprayed controls. No differences have been noted in the time of leaf fall.

Effect on fruit

With most varieties any increases in the amount of red color formed do not occur suddenly but come in gradually over a period of weeks. There is a change not only in the amount of pigment produced but also in the quality of the pigment formed. Thus the normal, natural red color of the Stayman is a dark red or brick red frequently admixed with varying amounts of yellow, green, and brown. The Stayman sprayed with thiocyanates, on the other hand, shows a brighter red with a coral tint. This difference is most readily observed by a comparison of sprayed and unsprayed fruits.

In addition to the changes in red coloration there are also changes in ground color. The dark green of the unsprayed fruit is replaced in considerable degree in the sprayed fruit by the lighter greens, yellow greens, and yellows ordinarily associated with more advanced maturity. This is especially true for varieties normally showing relatively small areas of red and correspondingly larger areas of green or yellow. When sprayed with thiocyanates, Golden Delicious and Grimes may be greenish yellow or full yellow rather than green when harvested. Spraying likewise tends to increase slightly the amount of blush sometimes found on these varieties. When Yellow Transparent was sprayed with sodium or calcium thiocyanate the stage for marketing was reached several days earlier than with unsprayed fruit, largely as a result of the hastening of the color change from green to yellow. Other maturity tests might be advisable, however, before picking such fruits for market.

Although changes in ground color would seem to indicate a hastening of maturity, a corresponding progress in softening does not appear to take place. No pressure tests have been made thus far, but general observations show a firmness of tissue probably equalling that of unsprayed fruit. More-

over, retention on the tree seems to be no less for the sprayed fruit and may be somewhat greater in certain varieties. Furthermore, storage records thus far indicate a superior keeping quality for the sprayed fruit. This might be anticipated, however, since it is generally recognized that fruit of high color is less subject to scald in storage than corresponding fruit of low color.

Finally, some varieties show still further changes in the sprayed fruit. Stayman, for instance, may show a smoother surface with less scarf skin, accompanied by a moderate reduction in the size and prominence of the lenticels, and a somewhat thickened skin. Under certain conditions Grimes has taken on a waxy, yellow-amber appearance when sprayed with nicotine thiocyanate.

Discussion

During the summer of 1939 some fifty trees were sprayed with a power-spray outfit using sodium thiocyanate, alone, at concentrations of 0.05, 0.1, or 0.2 per cent., respectively. Of these trees about thirty were located on the University Horticulture farm at Morgantown, and the remainder on the University Experiment Farm located at Kearneysville, in the eastern panhandle section near Martinsburg, West Virginia. Usually the first spray was of 0.1 per cent. concentration, and the following sprays were of 0.05, 0.1, or 0.2 per cent., respectively, depending upon the response as shown by the amount of foliage burn and color increase obtained. When only one spray was applied, particularly if this was applied rather late in the season, a concentration of 0.2 per cent. was usually employed. On the whole the results obtained at Kearneysville were not nearly as pronounced as those obtained at Morgantown. This difference in physiological response appears to be related to environmental factors, as several of the varieties tried were the same in each location.

One noticeable result of the thiocyanate spray is the difference in susceptibility to foliage burn, as shown by the different varieties. Thus the foliage of Delicious and Stayman is relatively resistant, whereas Golden Delicious and Rome are more readily injured by the spray. Color responses likewise differ with the different varieties. Stayman has responded rather better than Delicious in color effect; and Jonathan, a variety usually carrying good color in this region, nevertheless gave considerably better response than others of lower color such as Spy. It is easily possible that part of the differences experienced thus far are to be attributed to inherent individual factors since it has been observed that different trees of the same variety in the same row may show considerable variation in response to the treatment. It seems probable, however, that there are general varietal differences also.

Another feature resulting from the thiocyanate spray, and easily apparent, is the larger number of fruits showing some color and the greater amount of color on the inside branches in positions of poorer exposure to

light. With some varieties and in certain seasons normal coloration appears to be fairly good, as seen on the tree, when in reality the color is confined almost entirely to a shell of exposed fruits on the outer branches. When sprayed with thiocyanate such trees show an increased distribution of color underneath on the interior branches This does not mean that light is unessential to color development on the sprayed fruits. On the contrary, light is a very important factor either with or without thiocyanate spray, but the latter aids in the production of color for any given conditions of exposure.

As stated earlier the increase in red color following thiocyanate sprays usually does not take place suddenly, but comes in gradually. The foliage, on the other hand, responds somewhat more rapidly. Consequently, it has been possible to gauge the concentrations and number of successive sprays desirable in any given instance as much (or more) by the leaf response as by the effect on the fruit. The thiocyanate ion appears to be a rather powerful physiological agent which affects both foliage and fruit. Further, if leaves only are sprayed and the fruits protected, or if fruits only are sprayed and the leaves protected, a color response will be obtained in the fruit in either case, but one less pronounced than where both fruit and foliage are exposed to the action of the chemical agent.

The thiocyanate spray has been applied in combination with other common spray materials including summer-strength lime sulphur, flotation sulphur, arsenate of lead, and bordeaux mixture, and appears to be compatible with each. Hand-spray trials of sodium thiocyanate *vs.* potassium thiocyanate and of sodium thiocyanate *vs.* calcium thiocyanate, in the same percentage concentration, indicated no pronounced differences in the color response obtained. More recently additional trials on similar exposures of the same tree gave no appreciable differences between solutions of equivalent thiocyanate ion concentrations of sodium, calcium, magnesium, and barium thiocyanates, respectively. Cuprous thiocyanate, and lead thiocyanate, however, were less effective in color response, possibly as a result in part of lower solubility. Ammonium thiocyanate is effective in increasing color but has the disadvantage of causing the production of small darkened areas or "pits" in the skin. These darkened areas, however, did not decrease keeping quality in storage. Among other organic thiocyanates, nicotine particularly, gave a fair color response in several varieties.

The work has not been of sufficient duration to warrant a statement of the probable effect of the thiocyanate treatment upon the bloom, set, or yield of the succeeding year's crop. In the hand-spray work individual limbs which showed moderate to severe foliage burn as a result of thiocyanate sprays in the summer of 1938, nevertheless carried good yields of fruit in 1939. It should be remembered, however, that only during the season just past have entire trees been subjected to this spray treatment, and conse-

quently evaluation of its effects on subsequent bloom and set of fruit must await further observation.

Summary

It has been found that soluble thiocyanates, particularly inorganic thiocyanates, used as a spray on apples during the growing season, exert a pronounced physiological effect on both foliage and fruit as follows: In the foliage the leaves are subjected to spray burn and to a chlorotic condition arising from the effect of the chemical on the green coloring matter of the plant. In the fruit the amount of red color occurring normally tends to be increased and the green ground-color tends to be reduced or replaced by varying shades of yellow and yellow green.

The authors desire to express their indebtedness and appreciation to various members of the station staff, and particularly to Prof. R. S. MARSH of the Department of Horticulture, and EDWIN GOULD of the Department of Entomology, for generously providing facilities and cooperating in every possible manner with the work.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

1. DENNY, F. E. Hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 118–125. 1926.
2. ———————. Second report on the use of chemicals for hastening the sprouting of dormant potato tubers. Amer. Jour. Bot. **13**: 386–396. 1926.
3. ———————. Chemical treatments for controlling the growth of buds of plants. Ind. & Eng. Chem. **20**: 578–581. 1928.
4. DUNCAN, IRA J., and DUSTMAN, R. B. The anthocyanin pigment of the Winesap apple. Jour. Amer. Chem. Soc. **58**: 1511–1514. 1936.
5. DUSTMAN, R. B., and DUNCAN, I. J. Red color in apples. Science, n.s. **90**: 233. 1939.
6. SANDO, C. E. Coloring matters of Grimes Golden, Jonathan, and Stayman Winesap apples. Jour. Biol. Chem. **117**: 45–56. 1937.

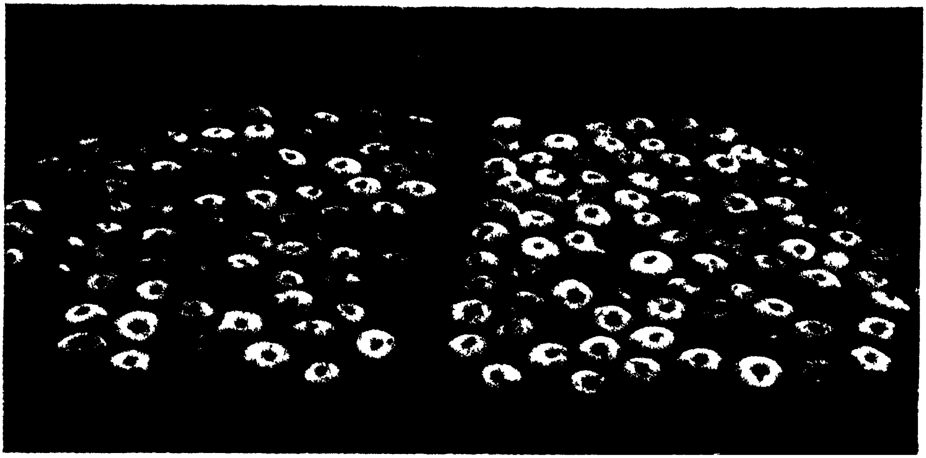


FIG. 1. Fruit from Delicious tree in Fig. 2. Control on right
Photographed Sept. 28, 1939

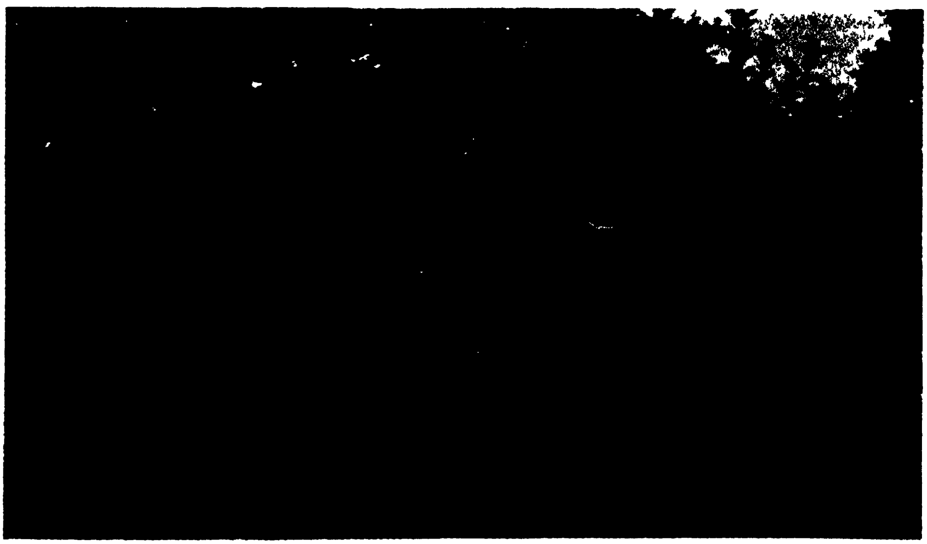


FIG. 2. Delicious, sprayed four times. Control limb with white tag in center
Photographed Sept. 6, 1939

DUSTMAN AND DUNCAN: THIOCYANATE SPRAYS

Reproduced from Kodachromes

DOES "C.P. GRADE" SUCROSE CONTAIN IMPURITIES SIGNIFICANT FOR THE NUTRITION OF EXCISED TOMATO ROOTS?

PHILIP R. WHITE

(WITH TWO FIGURES)

Ever since the early work of KOTTE (2) and ROBBINS (4) on the limited cultivation of excised roots there has persisted the conviction, formulated at that time, that growth of such roots is dependent on unknown as well as known factors. ROBBINS (5), ROBBINS and MANEVAL (7, 8) and later ROBBINS and V. B. WHITE (10) concluded that growth of excised corn roots was dependent on some unknown material not contained in the salts of Pfeffer's solution, glucose, water, dissolved gases, peptone, or autolyzed yeast. It has subsequently been shown that continued growth of excised roots of many plants other than corn can be obtained in a nutrient containing these same salts, brewer's yeast, and sucrose (12, 14) and that roots of tomato, sunflower, and pea can be grown in a similar salt solution plus cane sugar, thiamin, and glycine, or other amino acids (17). ROBBINS and SCHMIDT (9), and FIEDLER (1) nevertheless return to this idea of unknown essentials in discussing recent results obtained with tomato roots and postulate the existence of such unknowns in the carbohydrates used.

This interpretation may, of course, be sound. But it appears not to be based on actual evidence. It should, however, be possible to test this question. If the growth obtained with certain sugars is attributable to the presence of impurities, as suggested, then progressive removal of these impurities should result in progressively poorer and poorer growth. To obtain evidence bearing on this question, a sample of sucrose *purissima* was obtained from the U. S. Bureau of Standards in Washington, D. C.[1] This is their "Standard Sample no. 17" prepared for use in molecular weight determinations, calorimetric studies, etc. Its designated properties are: specific rotation

$$[\alpha]\frac{20^\circ\text{ C.}}{546.1\text{ m}\mu} = 78^\circ.342 \qquad [\alpha]\frac{20^\circ\text{ C.}}{589.25\text{ m}\mu} = 66^\circ.529$$

when C = 26 gm. in 100 ml.; heat of combustion 3941 calories per gram weight in air; lot no. 3854; moisture less than 0.003 per cent.; ash, 0.003 per cent.; reducing substances estimated as invert sugar, 0.002 per cent. This sugar is prepared by repeated crystallization. The Bureau of Standards states: "This material is the result of many years experimentation and experience in handling and in methods of preparation.

[1] I am indebted to DR. EUGENE PACSU, Professor of Organic Chemistry in Princeton University and specialist in the chemistry of sugars, for advice and counsel on this subject.

The impurities have been reduced to the lowest possible point consistent with the issuing of a standard sample" (3). Spectroscopic examinations carried out in The Rockefeller Institute laboratories in New York by Dr. GEORGE I. LAVIN indicate that the inorganic impurities are largely calcium with some magnesium. Traces of calcium or magnesium are, of course, without significance (11). While an ash content of 0.003 per cent. still represents a total ash impurity of 0.6 mg. per liter of nutrient, it does not appear feasible to push the purification further at present. This paper gives the results of experiments designed to compare the effectiveness of this highly purified sucrose with that of "C.P. grade" sucrose such as was used in all earlier studies, with commercial cane sugar available in grocery stores, and with Pfanstiehl's "C.P. grade" dextrose, considered by ROBBINS and BARTLEY (6) and by ROBBINS and SCHMIDT (9) as being equally effective with sucrose as a carbohydrate source for tomato roots.

Experimentation

Roots of this laboratory's standard clone were used for these tests. They had been grown for 301 passages in the yeast nutrient earlier employed and then for three passages in a completely synthetic (glycine-thiamin) nutrient (17), which is now used as a standard in all work. The nutrients tested contained the standard salts and accessory salts discussed elsewhere (16), 3 p.p.m. glycine (17) and 0.1 p.p.m. thiamin (15), and carbohydrate. Twenty flasks contained the usual 2 per cent. Pfanstiehl "C.P. grade" sucrose and represented the standard control. Twenty contained 2 per cent. commercial cane sugar purchased in a local grocery, 20 contained 2 per cent. Bureau of Standards standard-sample sucrose as described above, 20 contained 2 per cent. Pfanstiehl "C.P. grade anhydrous" dextrose of the same make and grade as that used by ROBBINS and BARTLEY (6) and ROBBINS and SCHMIDT (9) and, since a 2 per cent. solution of dextrose has an osmotic value twice that of a 2 per cent. sucrose solution, a fifth set of 20 was prepared containing 1 per cent. dextrose, isotonic with the sucrose solutions. Each flask contained a single root, the inocula having been carefully selected to insure uniformity. Roots were grown in these five solutions through three consecutive passages and the increment rates, numbers of branches, and general appearance compared. The series was repeated once. The results of these 600 cultures are shown in figures 1 and 2.

The numerical growth indices for the three samples of sucrose did not differ consistently or significantly either from sample to sample or from passage to passage in solutions containing the same sample, although the average index for store sugar and for Bureau of Standards sucrose was in each case about 10 per cent. greater than the control (18). Nor did the average number of branches formed differ significantly. The growth habits, however, did differ

somewhat. Roots in Pfanstiehl's "C.P. grade" sucrose and in grocery store sugar were almost identical in appearance, being rather thick, white and bent. The average maximum length of their branches was 10 mm. and 9 mm., respectively. Those grown in the Bureau of Standards sucrose were somewhat more slender and flexible, not bent, with somewhat shorter branches (7 mm.

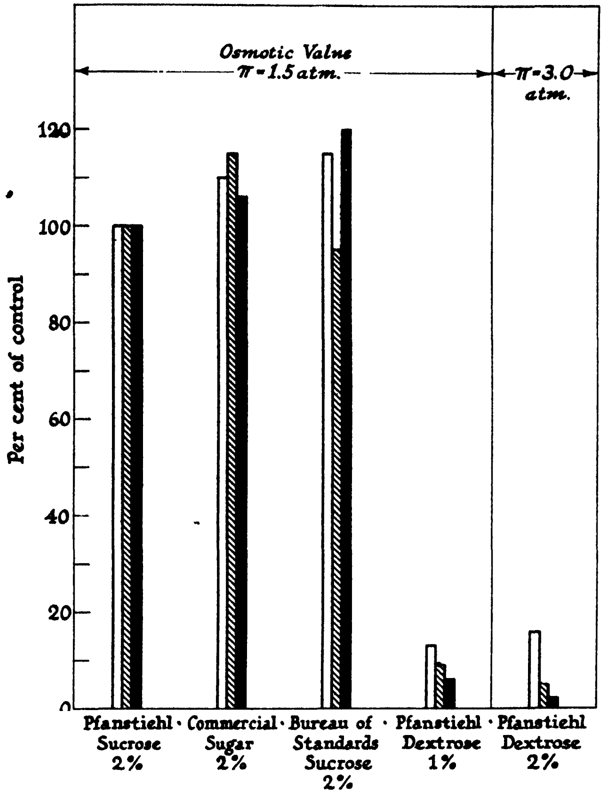


FIG. 1. Numerical indices of growth of excised tomato roots in various sugar solutions as indicated. Open columns represent the first passage, hachured columns the second passage, and solid columns the third passage in the experimental solution.

average maximum) but more regular habits. While experience has shown that, in general, thickened roots of the type formed in the first two sugars result from slightly injurious cultural conditions, the difference was not sufficiently marked to be of certain significance. Roots grown in dextrose solutions, on the other hand, were definitely inferior to those grown in any of the sucrose solutions (18). This last finding is contrary to the conclusions of ROBBINS and BARTLEY (6) and ROBBINS and SCHMIDT (9) and supports the earlier conclusions of the writer (13).

Fig. 2. The two roots at the left were grown in 2 per cent. store sugar, the set next to them in 2 per cent. Pfanstiehl "C. P. grade" sucrose, those in the middle in 2 per cent. Bureau of Standards sucrose, those next in 2 per cent. Pfanstiehl "C. P. grade" dextrose and those at the far right in 1 per cent. Pfanstiehl "C. P. grade" dextrose. The photograph was taken at the end of three passages in each solution. (Photograph by J. A. Carlile.)

Discussion and conclusions

From the data presented here, it is clear that growth in the most highly purified sample of sucrose was slightly superior to that obtained in the less pure samples. Pfanstiehl's "C.P. grade" sucrose may contain impurities which affect the growth of excised tomato roots, but these impurities certainly do not stimulate elongation. They tend rather to retard it. The Bureau of Standards sucrose contained about 35/1000 as much ash as did Pfanstiehl's "C.P. grade," yet supported slightly more satisfactory growth than did the latter. A differential of 35 : 1000 in known ash content had little effect on growth and that opposed to the effect expected from the theories cited above. Pfanstiehl's "C.P. grade" sucrose does not appear to be superior in this respect to the particular sample of grocery store sugar used in these tests, although no evidence has been collected as to the relative reliabilities of sugar from these two sources. The facts can be reconciled with the idea of significant impurities only if we assume that both pure and impure sugars contained the same impurities at concentrations giving the same effects. Either the postulated impurities were organic and were not fractionated by

the recrystallization process, or else they were present in quantities greatly in excess of the optimum, yet were not toxic at these high concentrations. These are rather large assumptions to make without supporting evidence. And the facts are equally well in agreement with the alternative and much simpler assumption that significant impurities were not present in these materials.

It is, therefore, concluded that the best evidence at present available does not indicate the existence in "C.P. grade" sucrose of impurities necessary for, or clearly beneficial to, the growth of excised tomato roots.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. FIEDLER, H. Die pflanzliche Gewebe- und Organkultur. Zeitschr. Bot. **33**: 369–416. 1938.
2. KOTTE, W. Kulturversuche mit isolierten Wurzelspitzen. Beitr. allg. Bot. **2**: 413–434. 1922.
3. NATIONAL BUREAU OF STANDARDS, Washington, D. C. Letter of February 13, 1939.
4. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
5. ———. Effect of autolized yeast and peptone on growth of excised corn root tips in the dark. Bot. Gaz. **74**: 59–79. 1922.
6. ———, and BARTLEY, MARY A. Use of dextrose by excised tomato roots. Science n.s. **86**: 290–291. 1937.
7. ———, and MANEVAL, W. E. Further experiments on growth of excised root tips under sterile conditions. Bot. Gaz. **76**: 274–287. 1923.
8. ———, and ———. Effect of light on growth of excised root tips under sterile conditions. Bot. Gaz. **78**: 424–432. 1924.
9. ———, and SCHMIDT, MARY BARTLETT. Growth of excised roots of the tomato. Bot. Gaz. **99**: 671–728. 1938.
10. ———, and WHITE, VIRGINIA B. Limited growth and abnormalities in excised corn root tips. Bot. Gaz. **98**: 209–242. 1936.
11. WHITE, P. R. Concentrations of inorganic ions as related to growth of excised root tips of wheat seedlings. Plant Physiol. **8**: 489–508. 1933.
12. ———. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.
13. ———. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.

14. ————. Cultivation of excised roots of dicotyledonous plants. Amer. Jour. Bot. **25**: 348–356. 1938.
15. ————. Vitamin B_1 in the nutrition of excised tomato roots. Plant Physiol. **12**: 803–811. 1937.
16. ————. Accessory salts in the nutrition of excised tomato roots. Plant Physiol. **13**: 391–398. 1938.
17. ————. Glycine in the nutrition of excised tomato roots. Plant Physiol. **14**: 527–538. 1939.
18. ————. Sucrose *vs.* dextrose as carbohydrate source for excised tomato roots. Plant Physiol. **15**: 355–358. 1940.

BRIEF PAPERS

SUCROSE VS. DEXTROSE AS CARBOHYDRATE SOURCE FOR EXCISED TOMATO ROOTS

PHILIP R. WHITE

In early studies on the cultivation of excised roots of wheat (3) carbohydrate was supplied in the form of "C. P. grade" dextrose (Pfanstiehl) at a concentration of 2 per cent. This choice was based on ROBBINS's earlier studies on roots of corn and was made without personal experimentation. Since the results seemed to be satisfactory, and interest at that time centered rather around other problems, no attempt was then made to determine the relative merits of other sugars. When, in 1932, attention was turned from wheat to tomato, comparably satisfactory results were not at first obtained. A survey of carbohydrate sources was made to determine if dextrose, shown to be satisfactory for wheat, might be surpassed by some other sugar. The carbohydrates tested were: one pentose (l-arabinose); five hexoses (d-mannose, d-galactose, d-glucose, d-fructose, and i-inosite); three di-saccharides (sucrose, lactose, maltose); one tri-saccharide (raffinose); one polysaccharide (dextrin); and one unidentified mixture (honey). All of these except the mannose and maltose (both Kahlbaum) and the honey were Pfanstiehl "C. P. grade" products. They were made up in nutrients containing the standard salts and yeast extract of the solution earlier developed and were introduced at concentrations equimolecular with the 2 per cent. dextrose solution previously employed. The results of that test are summarized in table I. They

TABLE I

GROWTH OF EXCISED TOMATO ROOTS IN NUTRIENTS CONTAINING EQUIMOLECULAR SOLUTIONS OF VARIOUS SUGARS

(DATA COLLECTED IN DECEMBER, 1932, AND JANUARY, 1933)

SUGAR	MEAN GROWTH RATE MM./CULT./DAY	CONDITION
l-arabinose	None	Crooked, pale. No growth
d-mannose	0.3	Slender, white, bent. Good condition
d-galactose	0.3	Slender, brown, tips black. Poor condition
d-glucose	0.5	Swollen, brown, branched. Poor
d-fructose	0.5	" " " "
i-inosite	0.6	Slender, white, tips dead. Poor
sucrose	3.6	Slender, clean, white. Excellent
lactose	0.6	Slender, clean, white. Good condition
maltose	0.5	Slender, brown, tips black. Poor
raffinose	0.7	Slender, clean. Bases green. Good condition
dextrin	0.7	Slender, clean, white. Good condition
honey	None	Swollen, brown. Poor condition

represent an unequivocal demonstration of the superiority of sucrose under these experimental conditions over all of the other sugars examined. A concentration of 2 per cent. sucrose was subsequently found somewhat superior to the 4 per cent. equimolecular with the 2 per cent. dextrose used for wheat. Since the major interest at that time was not to investigate carbohydrate metabolism but rather to find conditions which would permit unlimited growth, the details of these experiments were not published and the question was passed over with the statement (1934) that "sucrose . . . was found experimentally to be superior to dextrose (for tomato)" (**4**, p. 587) and (1936) that "tomato requires sucrose, being unable to utilize dextrose under the conditions studied" (**5**, pp. 430–431).

These statements stood unchallenged until 1937 and 1938 when ROBBINS and BARTLEY (**1**) and ROBBINS and SCHMIDT (**2**) concluded on the basis of their own experiments that "tomato roots *are* able to assimilate dextrose." These authors used several strains of tomato roots, including one obtained from the present writer, and used several sources of sucrose and dextrose. Their results do not show dextrose to be superior to sucrose but do show that carbohydrate source was not a limiting factor in their experiments (**2**, table 14). They are not directly comparable with results obtained in this laboratory, since a different source of yeast, a different length of culture period, and a different method of evaluating growth were employed. They are, nevertheless, sufficiently at variance with the results obtained here to make a re-examination of the question desirable. No attempt has been made to repeat the work of these authors. My own earlier experiments have been repeated using a technique similar to that which was employed before, except for minor improvements introduced from time to time during the past six years.

The roots used were of this laboratory's "Standard Clone C," also tested by ROBBINS and BARTLEY (**1**) and ROBBINS and SCHMIDT (**2**), and were in the 304th passage at the beginning of the experiments. The control nutrient was the completely synthetic solution employed as standard since June 21, 1938. This nutrient regularly gives results identical with those obtained in the earlier yeast extract medium. It contains the six salts of a modified Uspenski solution, four accessory salts, 0.1 p.p.m. thiamin and 3 p.p.m. glycine, and 2 per cent. Pfanstiehl "C. P. grade" sucrose. Its osmotic value is $\pi = ca.$ 1.5 atm. Four solutions were compared with this, all identical except for the carbohydrate. These were made up with (1) 2 per cent. commercial sugar bought in a local grocery; (2) 2 per cent. Bureau of Standards specially purified sucrose (**6**); (3) 2 per cent. Pfanstiehl "C. P. grade anhydrous" dextrose as nearly identical with that used by ROBBINS and BARTLEY and ROBBINS and SCHMIDT as obtainable, and giving an osmotic value of $\pi = ca.$ 3.0

atm.; and (4) 1 per cent. Pfanstiehl "C. P. grade anhydrous" dextrose having an osmotic value the same as that of the sucrose solutions. Cultures were grown in the laboratory used for all of the writer's work of the past six years, in diffuse daylight. Twenty cultures were grown in each solution, the experiments were carried through three passages, and the entire series was repeated once. The results of these 600 cultures are presented in table II (see

TABLE II

RELATIVE GROWTH RATES OF EXCISED TOMATO ROOTS IN NUTRIENTS CONTAINING SUGARS OF VARIOUS SORTS, CONCENTRATIONS, AND DEGREES OF PURITY

SUGAR	INCREMENT AS PERCENTAGE OF CONTROL			
	PASSAGE			AVERAGE
	1	2	3	
Sucrose Pfanstiehl C. P. 2 per cent.	100	100	100	100
Sucrose Bureau of Standards 2 per cent.	115	95	120	110
Sucrose Commercial 2 per cent.	110	115	106	110
Dextrose Pfanstiehl C. P. 1 per cent.	13	9	6	9
Dextrose Pfanstiehl C. P. 2 per cent.	16	5	2	8

also 6, figs. 1 and 2). While growth in the three samples of sucrose was excellent and did not differ significantly from the control nor diminish consistently from passage to passage, that in both concentrations of dextrose was extremely poor—less than 10 per cent. of that in the control nutrient—and it decreased in 2 per cent. dextrose from 16 per cent. in the first passage to 2 per cent. in the third, and in 1 per cent. dextrose from 13 per cent. in the first passage to 6 per cent. in the third.

The result of this experiment thus agrees with that obtained in 1933. Under the experimental conditions used as standard in this laboratory for the past six years, sucrose is superior to dextrose as a source of carbohydrate for excised tomato roots. Since the experiments were carried out with the same brand and quality of sugars and with the same strain of roots as were used by ROBBINS and BARTLEY (1) and ROBBINS and SCHMIDT (2), the discrepancy

between the results of these authors and those here presented remains unexplained.

THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. ROBBINS, W. J., and BARTLEY, MARY A. Use of dextrose by excised tomato roots. Science n.s. **86**: 290–291. 1937.
2. ——————, and SCHMIDT, M. B. Growth of excised roots of the tomato. Bot. Gaz. **99**: 671–728. 1938.
3. WHITE, P. R. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid media. Plant Physiol. **7**: 613–628. 1932.
4. ——————. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.
5. ——————. Plant tissue cultures. Bot. Rev. **2**: 419–437. 1936.
6. ——————. Does "C. P. Grade" sucrose contain impurities significant for the nutrition of excised tomato roots? Plant Physiol. **15**: 349–354. 1940.

KUNDT'S RULE

G. MACKINNEY

The writer recently found (**2**) an exception to Kundt's rule, with dichloroethane and acetone as solvents for chlorophyll. EGLE (**1**) objects to dichloroethane on the ground that pheophytin has been formed. The objection cannot be sustained in the writer's experiments for two reasons; first, that with this solvent, freshly purified and distilled, chlorophyll solutions are quite stable, and show negligible change after several hours if kept in the dark. The evidence, both chromatographic and spectroscopic, (**3**) is conclusive against such a change. Second, had such a change occurred, it would have yielded a result predictable from Kundt's rule, because the pheopytin maximum is at a slightly longer wave length than that of chlorophyll.

The results in a series of solvents tested by EGLE are in no way challenged, nor need the point be belabored, but for the assumption of some writers that it is permissible to extrapolate results with organic solvents and extracted chlorophyll, in order to predict the situation in the leaf. Fortunately, active interest in the chloroplastin complex will soon preclude the necessity for discussion of the rule.

DIVISION OF FRUIT PRODUCTS
UNIVERSITY OF CALIFORNIA

LITERATURE CITED

1. EGLE, K. Über die Gültigkeit der Kundtschen Regel bei Chlorophyll. Sitz. Heidelberg Akad. Wiss. Klasse Math.-naturwiss. Abh. **1**: 19–30. 1939.
2 MACKINNEY, G. Applicability of Kundt's rule to Chlorophyll. Plant Physiol. **13**: 427–430. 1938.
3. ————. Some absorption spectra of leaf extracts. Plant Physiol. **13**: 123–140. 1938.

NOTES

New England Section.—The New England Section will hold its annual meeting at Dartmouth College, Hanover, New Hampshire, on May 10–11, 1940. These meetings again will offer the opportunity for plant scientists in the area to participate in the programs of discussion and to add to their lists of friendships. Dr. CHARLES J. LYON of Dartmouth will have charge of the arrangements.

In addition to the papers to be presented on other subjects, several workers in the field of growth physiology will participate in a symposium on Growth Control in Trees. The flexibility of the program is such that groups readily form to discuss informally problems of common interest.

The annual dinner will be followed by pictures and stories of life in the North Country. All plant physiologists in the New England states will be cordially welcome to share in the benefits and privileges of the meeting.

Western Section.—The summer meeting of the American Society of Plant Physiologists and the meeting of the Western Section will be held at Seattle, Washington, from Tuesday, June 18, to Saturday, June 22, 1940. The program will include three symposia:

1. Photosynthesis, jointly with the Botanical Society of America.
2. Aquatic Botany, also jointly with the Botanical Society of America.
3. Phosphate Nutrition, jointly with the Society of Soil Scientists.

Three half-day sessions are reserved for the reading of short papers, and also one for papers having a bearing on horticulture, jointly with the Society for Horticultural Science.

On Saturday, June 22, an excursion to the Oceanographic Laboratories of the University of Washington at Friday Harbor has been planned. This provides an excellent opportunity to see the beautiful San Juan Archipelago with its amazing growth of algae such as the large Nereocystis, in addition to the outstanding laboratories.

Southeastern Section.—It is gratifying to be able to report that the Southeastern Section of the A.S.P.P. has completed its permanent organization. In a meeting held in connection with the Association of Southern Agricultural Workers at Birmingham, Alabama, on February 7, 1940, permanent officers were elected for the year 1940–41. Dr. CLAIR L. WORLEY, the University of Georgia is chairman of the Section, and *ex-officio* member of the executive committee of the national organization; Mr. DAVID D. LONG, Director of Agricultural Research of the International Agricultural Corporation, Atlanta, Georgia, was elected vice-chairman; and Dr. THOMAS J. HARROLD, University of Georgia, was elected secretary-treasurer. These

officers will draw up a constitution and set of by-laws to be presented for consideration at the 1941 meeting.

The Southeastern Section will continue its meetings with the Association of Southern Agricultural Workers annually in February. It has become affiliated with this Association, therefore, and at a later time will present a request for modification of its territorial limits to conform to that of the Association. This would require the addition of three or four states to the territory of the Section.

A membership committee has been organized with Dr. GEORGE M. ARMSTRONG, Clemson College, chairman. It seems certain that a larger membership in A.S.P.P. in the South will result from the activities of the Section. The 1940 meeting was attended by 14 men representing seven states, Virginia, North Carolina, South Carolina, Georgia, Alabama, Mississippi, and Louisiana. Three scientific papers were read, one on carotene and vitamin C by O. A. LEONARD; one on nutrient deficiencies in pine seedlings by L. J. PESSIN; and one on Nielsen's substance B by C. L. WORLEY. Those in attendance were enthusiastic over the privileges and opportunities afforded by organization. We predict a very bright future for this Section.

Changes in Proofs.—At the Columbus meeting the A.S.P.P. finally took official action on the matter of excessive changes made by authors in their proofs. Such changes may be defined as eliminations, additions, or changes in wording or punctuation not found in the original manuscript as submitted. It has been ascertained by statistical study that a change of 2 per cent. of the lines is an average change, actually achieved in some entire numbers of the journal. Any more than this will be considered excessive.

The editor has been authorized to have the costs for excessive changes in the proofs added to the author's bill for reprints. The charge is 12 cents per line. In administering this regulation, very simple rules are desirable. Authors will be allowed an average change of one line per page, which is just slightly more than 2 per cent. The printer's records will be used to determine how many lines have been changed; and when the author's line changes exceed one per page of printed text, the excess changes at 12 cents per line will be added to the bill for reprints. The majority of authors will find that this regulation causes them no extra expense. The few who do not properly polish their manuscripts before submitting them, or who change their minds about interpretations before the papers can be printed, should pay for the extra work they demand.

Corresponding Members.—Election of corresponding members to the American Society of Plant Physiologists is strictly limited. In order to provide complete information concerning the present roster of Corresponding Members, the list is printed in full, with date of election.

FREDERICK F. BLACKMAN, University of Cambridge, 1932.

GOTTLIEB HABERLANDT, University of Berlin, 1934.

BOHUMIL NĚMEC, Charles University, Prague, 1935.

NICOLAI A. MAXIMOV, Saratov, 1935.

HENRY H. DIXON, Trinity College, Dublin, 1937.

ALFRED URSPRUNG, University of Freibourg, 1937.

SIR JOHN RUSSELL, Rothamsted Experimental Station, 1938.

Three other great plant physiologists have been elected, but have died since election. These are:

F. A. F. C. WENT, University of Utrecht, 1932; deceased 1935.

V. N. LUBIMENKO, Leningrad, 1934; deceased 1937.

HANS MOLISCH, University of Vienna, 1935; deceased 1937.

Program Committee.—The program committee for the seventeenth annual meeting at Philadelphia has been appointed by President JOHN W. SHIVE, as follows:

Dr. J. T. CURTIS, the University of Pennsylvania.

Dr. W. R. ROBBINS, New Jersey Agricultural Experiment Station.

Dr. WILLIAM SEIFRIZ, chairman, the University of Pennsylvania.

With this able leadership, and with cooperation of all members of the Society it should be possible to make this meeting a memorable occasion. Those who wish to participate in the sessions can aid the committee very materially by early submission of titles. If there is sufficient demand for time on the program, parallel programs of non-competing subjects will be arranged. Suggestions from the members are always welcome.

Frank Marion Andrews.—At the sixteenth annual meeting of the American Society of Plant Physiologists it was voted to dedicate one of the numbers of Plant Physiology for 1940 to Dr. FRANK MARION ANDREWS, who will celebrate the seventieth anniversary of his birth on July 27, 1940. Dr. ANDREWS was born at Vienna, a small village in Scott County, Indiana, on July 27, 1870. He was educated in the public schools of Indiana, and after completing his secondary education, he entered Indiana University, with which he has been identified for half a century. He received his A.B. degree at Indiana in 1894, and the A.M. in 1895. He became an assistant in botany at I. U. in 1894, a position he held until 1897, when he was given the rank of instructor. During his instructorship, 1897–1904, he took leave long enough to win his Ph.D. degree at Leipzig in 1902. He spent a summer at the Marine Biological Laboratory, Woods Hole, Massachusetts, in 1903. In 1904 he was promoted to an assistant professorship, a position he held until 1907. It was during this period that he met Miss MARIE OPPERMAN, a botany major at Indiana University, afterwards a student with Dr. MARGARET C. FERGUSON at Wellesley for an advanced degree. They were married on June 17,

1907. Dr. ANDREWS was promoted to an associate professorship in 1907, a rank which he held until 1922, when he was advanced to a professorship. This position he still holds, for he reaches the usual retiring age with one more year of service. It is unusual indeed for anyone to be identified with a single institution for so many years.

As a teacher, Professor ANDREWS has an enviable reputation as one of the best on the I. U. campus. His class work was always well organized, and went along so smoothly that students accomplished a large amount of work without strain. The spirit of his class rooms and laboratories was one of jovial comradeship in the work to be accomplished; and students loved and respected his sensitive feeling for how much and how well the work should be done. They could not bear to fail him; work, spiced with good humor and pithy admonitions, was pleasure even in oppressively hot summer weather. Students learned much besides the regular prescribed work.

His personality, his thorough knowledge of his subject and of other fields of interest, his unfailing sense of humor, his personal oversight of the preparation for work, his kindly personal interest in all students, bright or otherwise, and his keen desire to see each one succeed, are the elements of his success as a teacher. A student would not need to be told more than once that: "A college education for some, is a four-year attempt to keep from getting what one comes to college to get!" When the tension of work reached the danger point, a pleasant joke brought relaxation and renewed zest for labor.

Professor ANDREWS belongs to numerous scientific organizations, was president of the Indiana University chapter of Sigma Xi in 1916, is a fellow in the Indiana Academy of Science, which organization he served as vice president in 1920, and president in 1921. He belongs to the Soc. Linn. de Lyon. His interest in the American Society of Plant Physiologists ever since its organization has been demonstrated in many services rendered. In 1935 he was awarded the CHARLES REID BARNES life membership in recognition of his long and valuable service to plant physiology. In 1937 he demonstrated his abiding interest in the Society's welfare by becoming one of its patrons at the Indianapolis meeting. In a way, the Society represents to him a dream come true, in what it is accomplishing for the welfare of plant physiology and plant physiologists.

Among his priceless memories are the privileges of association with Dr. GEORGE J. PEIRCE, who was Assistant Professor at Indiana during the last two years of his assistantship in botany ('95–'97), and with the great leaders in Germany, Holland, and Italy during his European visits. After his degree had been awarded in 1902, he had visited Amsterdam in 1907, Leipzig in 1907–1908, the Naples Station in 1908, and Strassburg in 1908–1909. He occupied the Smithsonian table at Naples. The inspiration of tireless men who aided him and supplied his needs for research during these "Wander-

jahre" has lived in his heart and mind, and has helped to set the pattern of his life. More than a hundred contributions to the literature of plant physiology attest his devotion to research.

That he lives the principles of democracy has been demonstrated in his recent illness. None have been too humble to remember him. People in all walks of life, those on relief, day laborers, and janitors, as well as professors and bankers, rich and poor, regardless of color, have united in sympathetic interest in his welfare. Such tribute comes to those whose lives bear the stamp of truly democratic ideals.

The photographs reproduced in this issue of PLANT PHYSIOLOGY were prepared by the Shaw Studio, Bloomington, Indiana. The fireside portrait was enlarged from a small photograph taken by Mr. SWANSON, a photographer of the U. S. Navy. We appreciate the opportunity and privilege of reproducing them.

On behalf of the members of the American Society of Plant Physiologists, we extend congratulations and good wishes to Professor and Mrs. ANDREWS on the occasion of the seventieth birthday anniversary. We hope that all signs of ill health may vanish and that both may enjoy many more years together.

Minor Elements.—The following announcement has been sent to Plant Physiology by Mr. HERBERT C. BREWER, Director of the Chilean Nitrate Educational Bureau, Inc., 120 Broadway, New York City.

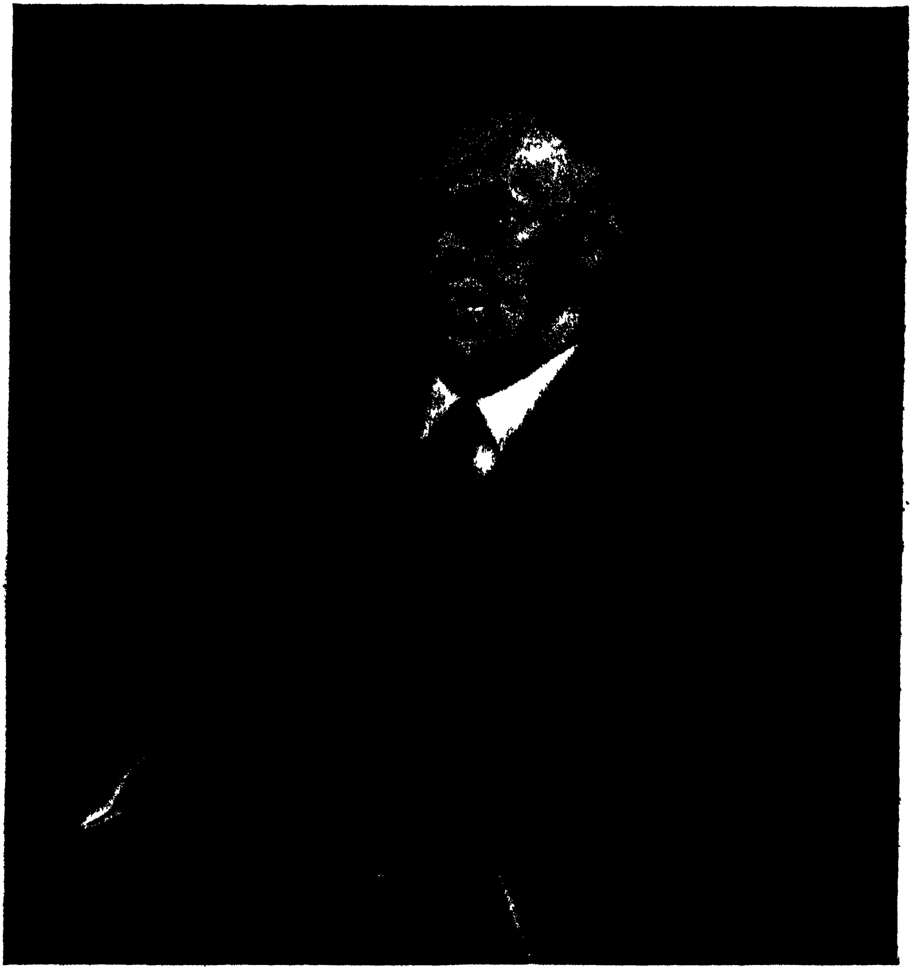
"The third edition of the *Bibliography of References to the Literature on the Minor Elements,* published Feb. 1, 1939, contained 4,628 abstracts and references, in a volume of 488 pages."

"Owing to its size, it is not practical to continue publication of complete editions of the bibliography, especially since the volume of material becoming available makes it desirable to publish more frequently. Accordingly Supplement No. 1 to be published shortly will contain about 700 abstracts and references, noted since publication of the third edition."

"It is planned hereafter to publish supplements at intervals of approximately one year."

"A botanical index is now available for the third edition, and is also being included in the supplement."

It is unnecessary to emphasize the importance of this announcement, or of the service rendered to plant physiologists and to agriculture generally by this program of publishing annual supplements to this important Bibliography. Every member of the American Society of Plant Physiologists should avail himself of the opportunity to obtain these supplements as they appear. The botanical index will add immensely to the value of the third edition. The inauguration of this high grade service to scientists reflects great credit upon the Bureau and its Director.



CHARLES FREDERICK HOTTES
JULY 8. 1870

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

CHARLES FREDERICK HOTTES

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 8, 1870

THIS NUMBER OF PLANT PHYSIOLOGY

IS DEDICATED TO

CHARLES FREDERICK HOTTES

IN CELEBRATION OF

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

JULY 8, 1870

CHARLES FREDERICK HOTTES
JULY 8, 1870

Vol. 15 No. 3

PLANT PHYSIOLOGY

JULY, 1940

STARCH FORMATION IN TOBACCO PLANTS DEFICIENT IN POTASSIUM[1]

DOROTHY DAY

Potassium is important in the growing of fine tobacco. Not only is it the nutrient removed from the soil in the greatest quantity, but it is essential for the vigorous growth of the plant (3). Potassium is also more important than any other element in producing good combustion of the dried leaf (1) and is necessary for choice aroma (5).

This value of potassium to the finished tobacco product has a curiously paradoxical aspect, for it has long been assumed that much of the importance of this element to any plant lay in its relation to carbohydrate synthesis or translocation. But the type of tobacco grown in the Connecticut Valley is low in carbohydrates, especially starch. Consequently, there is a special interest in the problem of the relationship of potassium to the formation of starch in tobacco plants.

It was the aim in this study, as in much recent work, to test the leaves soon after the effects of potassium deficiency had been definitely established but well before the plant had died from lack of this element. From preliminary trials with tobacco, it appeared that seedlings started in soil, transplanted to sand, and treated with a nutrient solution, could be grown normally with the usual elongation of the internodes; or that similar plants, treated in a like manner except for the absence of potassium, would show evidence of a lack of this element during the latter part of the vegetative period.

Methods

Tobacco (*Nicotiana tabacum*, var. Rosenberg)[2] was germinated and grown in quantity in sandy soil until the seedlings were about two inches tall with four small leaves, from 9 to 15 weeks depending upon the season.

[1] Contributions from the Department of Botany, Smith College, New Series, no. 5.

[2] These seeds were kindly supplied by the Connecticut Agricultural Experiment Station where the starch analyses were completed in the Department of Biochemistry. The writer is indebted to Dr. H. B. VICKERY and Dr. G. W. PUCHER of that department for their continued interest and advice.

Uniform seedlings were washed thoroughly to remove all soil, and single specimens were placed in pure quartz sand in glazed jars, each with a lateral drainage hole covered on the inside with absorbent cotton to prevent loss of sand. The complete nutrient solution was one that PUCHER[2] had found suitable for growing this strain of tobacco under similar conditions (table I). In the solution lacking potassium, the potassium phosphate was

TABLE I

COMPOSITION OF CULTURE SOLUTIONS, GRAMS PER LITER

SALTS	COMPLETE NUTRIENT SOLUTION	SOLUTION MINUS POTASSIUM
KH_2PO_4	0.442	0.0
$Ca(NO_3)_2 \cdot 4H_2O$	1.015	1.015
$MgSO_4$	0.126	0.126
$NaH_2PO_4 \cdot H_2O$	0.0	0.497
$Fe_2(C_4H_4O_6)_3$	Trace*	Trace*
H_3BO_3	Trace*	Trace*
$MnSO_4 \cdot 4H_2O$	Trace*	Trace*

* 0.5 p.p.m. of Fe, B, and Mn.

replaced by sodium phosphate. The continuous flow method of SHIVE and ROBBINS (19) was used, the drip being regulated to furnish about two liters of nutrient solution in twenty-four hours; this was sufficient until the last week of growth when the rate was increased. Four series of plants transplanted into sand cultures respectively in November (T_1), January (T_2), March (T_3), and May (T_4) were employed.

The plants were harvested toward the end of the vegetative period, 35–39 days after transplanting, when the control plants were about 35 inches tall. The samples were taken at 3:00 P.M. on a sunny day in January (T_1), March (T_2), April (T_3), and June (T_4). All leaves below the third node, counting down from the bud, were cut separately; these individual leaves were at once weighed and dried at 70°–80° C. and were weighed again. The dried leaves from corresponding nodes of several plants were grouped together and ground to a fine powder. Weighed samples of about 200 mg. were analyzed for starch according to the iodine precipitation method of PUCHER and VICKERY[2] (15) as slightly modified in previous similar work on peas. (2).

Results

The control plants grew normally while those starved of potassium showed the characteristic appearance resulting from a lack of this element (5, 12, 13). The former were tall with about twenty-five nodes per plant, while the latter were short with about sixteen nodes; consequently, a leaf at the twelfth node in one of these plants may be a basal one, or very near the bottom, while a leaf so numbered on a control plant may be near the middle.

The fresh weight per single leaf reached a maximum for all series and for both treatments in the control plants harvested in June (table II). In the plants with the complete nutrient solution, the fresh weight per leaf at corresponding nodes increased progressively through the season, whereas the plants starved of potassium varied little in this respect, and the leaves harvested in June were not as heavy as earlier ones. In most cases, for both treatments, there was an increase in weight from the younger, upper leaves down to the sixteenth or eighteenth node and then a decrease. The fresh weight per leaf was generally greater in the control plants than it was in the deficient plants; it was about twice as great for those harvested in January, and three times as much in March, April, and June.

The values for dry weight per one leaf are about one-tenth of those for the fresh weight (table II). The dry weight as percentage of fresh weight tends to decrease from the top to the base for all plants, and is generally greater in the control plants with the exception of the series harvested in March.

The percentage of starch is greater in all leaves of the control plants of the June series and in all except the topmost leaves of the April series (table II). In the March series, however, nearly all the leaves from the potassium deficient plants contained a higher concentration of starch than their corresponding controls, the values in a few cases being unusually high for this type of tobacco. The general trend for both treatments seems to be an increase in percentage of starch from the younger to the older leaves and then with a few exceptions a steady decrease from about the eighth leaf. In the control plants the proportion of starch was greater the later in the season the plants were grown. The reverse was true for those without potassium.

The amount of starch in one leaf was approximately the same in the top leaves of all plants, generally being 1.0 mg. or less at the fourth or fifth node (table II). In the leaves of the control plants, it reached its maximum at or near the sixteenth node and then decreased to the base. In the plants deprived of potassium, this maximum was at about the eighth leaf. The amount of starch per leaf was generally more in the control plants than in those starved of potassium except for the upper leaves of those harvested in March. The maximum amount of starch in a leaf of the control plants was twice that of the corresponding leaf of the experimental plants for the series harvested in March, eight times as much for the April series, and eleven times as much for the June series.

Discussion

Potassium is obviously concerned in the growth of the tobacco plant since the control plants grew normally while those without potassium were

TABLE II

FRESH WEIGHT, DRY WEIGHT, AND STARCH IN TOBACCO LEAVES

SERIES	T_2—MARCH				T_3—APRIL				T_4—JUNE			
NODE FROM TOP	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF	FRESH WEIGHT PER ONE LEAF	DRY WEIGHT PER ONE LEAF	STARCH PERCENTAGE OF DRY WEIGHT	STARCH PER ONE LEAF
A COMPLETE NUTRIENT SOLUTION												
	gm.	*gm.*	%	*mg.*	*gm.*	*gm.*	%	*mg.*	*gm.*	*gm.*	%	*mg.*
4	0.22	0.03	2.09	0.63	0.23	0.03	2.39	0.65	0.19	0.03	...[a]	...[a]
5	0.35	0.05	3.24	1.62	0.47	0.08	1.26	0.97	0.29	0.05	...[a]	...[a]
6	...	...	...	...	...	...			0.55	0.01	1.95	1.87
7	0.90	0.13	2.23	2.90	1.20	0.19	2.70	5.21	1.06	0.18	1.95	3.43
8	1.59	0.20	2.84	5.68	1.95	0.28	2.84	8.04	1.61	0.24	5.80	14.09
10	...	...	...	...	...	...	...	.	3.79	0.54	4.11	22.03
11	2.82	0.30	2.62	7.86	4.81	0.65	3.62	23.39	...	...	...	...
12	...	...	...	...	...	...	...	...	6.36	0.80	4.50	36.00
15	6.13	0.59	2.37	13.98	...	...	...	...	...	...	...	...
16	...	...	...	...	8.70	0.89	3.63	32.16	...	...	...	...
17	...	...	...	...	...	...	...	...	10.73	1.11	3.50	38.85
19	...	...	...	...	...	...	...	...	...	...	...	...
21	...	...	...	...	9.10	0.78	2.75	21.45	...	...	...	...
22	...	...	...	...	7.77	0.63	2.47	15.56	5.73	0.49	1.07	5.28
B SOLUTION MINUS POTASSIUM												
4	0.17	0.04	2.83	1.00	0.16	0.02	3.23	0.68	0.15	0.02	...[a]	...[a]
5	0.34	0.06	4.43	2.44	0.36	0.05	1.70	0.87	0.29	0.03	...[a]	...[a]
6	0.54	0.09	5.97	5.07	...	...	...	...	0.56	0.06	1.21	0.78
7	0.76	0.12	3.28	4.03	1.03	0.13	2.81	3.60	0.80	0.08	0.68	0.56
8	0.88	0.13	5.46	7.26	1.23	0.15	2.16	3.13	1.09	0.12	2.92	3.50
10	...	...	...	...	...	...			1.37	0.14	0.82	1.16
11	1.35	0.18	3.56	6.51	2.21	0.24	1.42	3.44	...	...	...	...
12	...	...	...	...	...	...	...	...	1.20	0.12	0.25	0.29
15	2.17	0.23	1.66	3.85	...	...	...	...	...	...	...	...
16	...	...	...	...	3.49	0.35	1.17	4.12	...	...	...	...
19	...	...	...	...	2.38	0.23	0.29	0.65	...	...	...	...

[a] Too little starch to test.

stunted and exhibited other deficiency symptoms. Furthermore, the control plants displayed a seasonal response by an increase in weight from January to June while the rate of development of the plants lacking this element was nearly independent of the time of year.

The control plants had more leaves which were both larger and heavier than did those deprived of potassium. This result agrees with the writer's previous work on peas and with most of the earlier reports (**2**). WARNE (**22**) also found that addition of potassium was associated with greater number and size of leaves in the beet. He suggests that increase of this element is accompanied by greater water content of the leaf, by more cell extension, by larger cell size, and by larger leaves. GREGORY and his co-workers have found increased water content in leaves with less potassium (**7, 8, 9**). STANESCU (**20**) associates high water content with synthesis of carbohydrates, a point that will be considered later. Although both the fresh and dry weights of these tobacco leaves were generally greater when potassium was furnished, the relationship of dry weight to fresh weight is the important point that must be considered. The evidence here was not conclusive, but the general trend was toward a greater percentage of dry matter and less water in the control plants. Also, in all leaves the proportion of dry matter was greater at the top of the plant than in lower leaves, that is, there was relatively less water in the upper, younger parts where available potassium generally accumulates. With peas, the dry weight per leaf also increased toward the top of the plant. However, as PHILLIPS, SMITH, and HEPLER (**14**) state, "It is true that decreased water content is found frequently associated with potassium deficiency, but it is also possible to find plants showing definite symptoms of potassium deficiency in which no significant differences in water content occur." Consequently, agreement is with them, rather than with SCHMALFUSS (**18**), in their conclusion that "the effect of potassium on the water relations of the plant is very important; but that this is its only function [as indicated by SCHMALFUSS] seems hardly compatible with the highly specific nature of the potassium requirement." ECKSTEIN (**4**) also has called attention to several critical aspects of this problem but concluded that there is still much to be done on the effect of potassium on the water economy of plants.

An indication for the need of potassium in the formation of carbohydrates is seen in the fact that, in each series, the maximal amount of starch in leaves of the control plants was greater than the maximum in the leaves of those deprived of potassium. This evidence, however, is not as conclusive as seemed to be the case in the work with peas (**2**). The amount of starch was generally more in the control tobacco plants than in those without potassium in most of the two later series, but there was a certain discrepancy in some leaves of the earlier series. RUSSELL (**17**) finds that

deficiency of potassium greatly reduces the rate of assimilation of carbon dioxide in barley. Similarly, WHITE (23) concludes that potassium starvation results in a low rate of assimilation in Lemna, but he also finds that it induces high starch content and high dry weight per unit area. As in the writer's work on peas, the lower tobacco leaves, but not the lowest, showed the greatest differences between the amount of starch in the control plants and that in the potassium deficient ones. When there is such a deprivation in barley, according to GREGORY and BAPTISTE (7), the sugar level consistently falls with the age of the leaf. All the uppermost tobacco leaves were more or less similar in storage of starch, and potassium is generally considered to be mobile, going toward the top, so it is possible that any available potassium may have moved upward and created similar conditions there in all the very young leaves with similar results as evidenced by like size, dry weight, and storage of starch.

With more daylight later in the season, the greater amount of starch in the leaves of the control plants is not unexpected, but this observation may raise the issue as to whether the carbohydrate activity is a cause or a result of better vegetative growth at this time. Since the reverse is true in the leaves of the plants starved of potassium, the question is raised whether or not these plants may be using carbohydrates for growth rather than merely for starch storage.

Curves plotted for each series to show the dry weight per leaf and the starch per leaf for all the plants grown at the same time with the same treatment are all closely alike for the control plants, although there is a slightly greater increase in starch than in dry weight as the season progresses. In the plants starved of potassium, the growth curves are less alike, as are the starch curves, and the two sets are distinctly unlike each other.

The greatest dry weight is not always observed in the leaf which is richest in starch. The leaf which shows the maximal percentage of starch is usually situated a few nodes higher than the leaf that shows maximal dry weight. It appears, therefore, that there are a few nodes at which, although the leaves are not yet fully grown, maximal starch storage is taking place. The most active photosynthesis thus occurs in these younger leaves. These nodes are a little farther apart in the plants deprived of potassium. Does this mean that the carbohydrate is being used for growth, with less storage, in the control plants? And is the carbohydrate stored rather than being used for growth in the potassium deficient plants?

Undoubtedly, these questions are closely related to the normal growth of the plant, and recent evidence indicates that a proper balance between potassium and nitrogen is important in this connection. GASSNER and GOEZE (6) state that assimilation is poorer with lack of balance of these

two elements than it is with the same degree of deficiency of both elements. HOFFER (10) and PHILLIPS, SMITH, and HEPLER (14) have reviewed the pertinent literature on this phase of the problem. The latter authors found that low potassium supply while there is a high level of nitrogen depresses growth and reduces the amount of starch in stems, but that with a low nitrogen supply "potassium deficiency actually improves growth slightly" and results in more starch in the stems. Similarly, the writer's work with peas showed definitely less starch in those plants grown without potassium, but this was at a high level of nitrogen with both nitrate and ammonium salts present. Also, TURCHIN (21) believes that potassium is more essential for the utilization of ammonium salts than for nitrates. Hence, the results in the work with peas may be partially due to an excess of nitrogen in the ammonium form, and potassium may not be as important in the storage of starch as appeared at that time. WHITE (24), working with the tomato, concludes that "The observed effects of potassium deficiency on fruiting are those also associated with carbohydrate deficiency relative to nitrogen supply."

RICHARDS (16) believes that potassium is not essential to protein synthesis, but that it is necessary to maintenance of the protoplasmic complex.

Pertinent to the problem of the storage of carbohydrates is the suggestion of JAMES and CATTLE (11) that potassium is not part of the diastase molecule, but that it is likely to act as a catalyst in the building up of the diastase complex. Similarly, WHITE (23) believes that a major rôle of potassium is regulation of the carbohydrate metabolism through control of the starch-sugar balance by activation of amylolytic activity. It is quite possible that more information on this point might help to explain some of the discrepancies in the literature, especially those in which there is conflicting evidence as to the effect of potassium on the presence or storage of the different carbohydrates.

It seems highly probable that PHILLIPS, SMITH, and HEPLER (14) are correct in suggesting that "the function of the element in question may well be indirect."

Summary

Plants of tobacco were grown from seedlings in sand culture with a complete nutrient solution and with a similar solution in which potassium phosphate was replaced by sodium phosphate. Leaves were cut separately, and the fresh and dry weights were ascertained. Quantitative determinations of starch were made by the iodine precipitation method of PUCHER and VICKERY.

The control plants grew normally with more and larger leaves having greater fresh weight and dry weight than those deprived of potassium. The proportion of dry matter in the leaves was greater in the control plants

and decreased from the top toward the base for all plants, that is, decreased water content was found associated with potassium deficiency.

An apparent correlation between the supply of potassium and the capacity of the plant to store starch was noted, inasmuch as the maximal amount of starch in the leaves of the control plants in each series was greater than the maximum in the leaves of the plants deprived of potassium.

Department of Botany
Smith College
Northampton, Massachusetts

LITERATURE CITED

1. Anderson, P. J., Swanback, T. R., and Street, O. E. Potash requirements of the tobacco crop. Connecticut Agr. Exp. Sta. Bull. 334. 1932.
2. Day, Dorothy, and Comboni, Silvia. Effects of potassium deficiency on the formation of starch in *Pisum sativum*. Amer. Jour. Bot. **24**: 594–597. 1937.
3. Donaldson, R. W. Quality tobacco in Massachusetts. Jour. Amer. Soc. Agron. **23**: 234–241. 1931.
4. Eckstein, Oscar. Effect of potash manuring on the production of organic matter. Plant Physiol. **14**: 113–128. 1939.
5. ————, Bruno, Albert, and Turrentine, J. W. Potash deficiency symptoms. Verlagsges. für Ackerbau M.B.H. Berlin. 1937.
6. Gassner, G., and Goeze, G. The importance of the potassium-nitrogen ratio in the soil for assimilation, chlorophyll content and transpiration of young cereal plants. Zeitschr. Pflanzenernähr., Düngung Bodenk. **36**A: 61–85. 1934.
7. Gregory, F. G., and Baptiste, E. C. D. Physiological studies in plant nutrition. V. Carbohydrate metabolism in relation to nutrient deficiency and to age in leaves of barley. Ann. Bot. **50**: 579–619. 1936.
8. ————, and Richards, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. Ann. Bot. **43**: 119–161. 1929.
9. ————, and Sen, P. K. Physiological studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. Ann. Bot. N.S. **1**: 521–561. 1937.
10. Hoffer, G. N. Potash in plant metabolism. Deficiency symptoms as indicators of the rôle of potassium. Ind. & Eng. Chem. **30**: 885–889. 1938.

11. JAMES, W. O., and CATTLE, M. Studies of the physiological importance of the mineral elements in plants. VII. The effects of potassium and chloride ions on the diastase of broad bean leaves. New Phytol. **34**: 283–295. 1935.

12. MCMURTREY, J. E., JR. Distinctive effects of the deficiency of certain essential elements on the growth of tobacco plants in solution cultures. U. S. Dept. Agr. Tech. Bull. 340. 1933.

13. ————. Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. **4**: 183–203. 1938.

14. PHILLIPS, T. G., SMITH, T. O., and HEPLER, J. R. Some effects of potassium and nitrogen on the composition of the tomato plant. New Hampshire Agr. Exp. Sta. Bull. 73. 1939.

15. PUCHER, G. W., and VICKERY, H. B. Determination of starch in plant tissues. Ind. & Eng. Chem., Anal. Ed. **8**: 92–97. 1936.

16. RICHARDS, F. J., and TEMPLEMAN, W. G. Physiological studies in plant nutrition. IV. Nitrogen metabolism in relation to nutrient deficiency and age in leaves of barley. Ann. Bot. **50**: 367–402. 1936.

17. RUSSELL, R. S. Physiological studies in plant nutrition. IX. The effect of mineral deficiency on the fructosan metabolism of the barley plant. Ann. Bot. N.S. **2**: 865–882. 1938.

18. SCHMALFUSS, K. Das Kalium: Eine Studie zum Kationenproblem im Stoffwechsel und bei der Ernährung der Pflanze. Freising—München. F. P. Datterer & Cie. 1936.

19. SHIVE, J. W., and ROBBINS, W. R. Methods of growing plants in solution and sand cultures. New Jersey Agr. Exp. Sta. Bull. 636. 1938.

20. STANESCU, PAUL P. Daily variations in products of photosynthesis, water content, and acidity of leaves toward end of vegetative period. Amer. Jour. Bot. **23**: 374–379. 1936.

21. TURCHIN, F. V. The rôle of potassium and phosphorus in the assimilation of various forms of nitrogen by plants. Zeitschr. Pflanzenernähr., Düngung Bodenk. **44**: 65–83. 1936.

22. WARNE, L. G. G. Effect of potassium supply on the water relations of foliage leaves. New Phytol. **35**: 403–417. 1936.

23. WHITE, H. L. The interaction of factors in the growth of Lemna. 7. The effect of potassium on growth and multiplication. Ann. Bot. **50**: 175–196. 1936.

24. ————. Observations of the effect of nitrogen and potassium on the fruiting of the tomato. Ann. Appl. Biol. **25**: 20–49. 1938.

RELATION OF pH TO GROWTH IN CITRUS

A. R. C. HAAS

(WITH TEN FIGURES)

Introduction

In a discussion of pH for healthy growth in citrus it was pointed out (**2**) that in culture solutions, growth was relatively poor at pH values above 8.0. For several years the problem of reaction and the growth of citrus has been studied under controlled conditions and in every case the conclusion has been reached that acidity is essential for prolonged health in the trees. A prerequisite, then, to healthy growth was the fact that during some period in the growth of the tree, it was necessary that acid conditions prevail.

Citrus, in California at least, is assumed to have a wide range of tolerance as regards pH. In contrast to the excellent groves found on soils as acid as pH 5.4 in Florida (**1**), it is concluded that equally fine groves in California may be found on soils with pH values of 8.5 or higher. According to certain recommendations, it is desirable to alter the pH of a soil only when the pH value exceeds 8.5 or is less than 5.0.

The degree of acidity or alkalinity of soils, such as is commonly encountered, is considered unimportant as regards any direct effect on the growth of citrus. The view is also frequently taken that pH determinations in solution cultures have little or no direct bearing on soil analysis or the growth of citrus in orchards.

In view of the wide divergence in pH values found in healthy groves in Florida and in California, it is difficult to reconcile the conclusions drawn from solution cultures with those obtained in the field. At first glance, it appears that results obtained in controlled artificial solution cultures should be severely discounted or should be considered as not being applicable in the field.

It should be stated, however, that pH determinations as carried out in commercial laboratories in southern California are commonly made upon a water suspension of dried soil, the dilution ranging from 1 part of soil in 1 part of water to 1 part of soil in 10 parts of water, the 1 to 5 ratio predominating. The pH values of these extracts are then determined by indicator or electrical methods. The use of rapid field tests in citrus groves has not met with much favor, largely because of the lack of experience in conducting such tests.

In solution cultures, the pH is determined by the indicator or electrical methods and the determination is simple and accurate. In soil cultures or under field conditions, the determination of pH has left much to be desired. Recently, the use of the speartype electrode (**6**) has brought greater ac-

curacy in the determination of pH in soils and as a result it becomes possible to compare more favorably the results obtained in solution cultures with those obtained in soil cultures or in the field.

The purpose of this paper is to emphasize the importance of pH for healthy growth in citrus and to show the results obtained when certain ranges of pH were used in solution cultures. Various sources of nitrogen were employed, in conjunction with ranges of pH, in the growing of citrus in culture solutions. Equally important are the soil cultures with citrus, in which the pH of the soil and the sources of nitrogen are related to growth. These studies then permit comparisons of the pH requirements of citrus in solution cultures with those found in soil cultures. The pH of soils as they occur under field conditions in the orchard will be considered in another report. In this way, evidence will be produced from several sources to show that when citrus trees are healthy they are growing primarily in an acid rather than in an alkaline medium and that the degree of acidity at which growth is best, is more acid than is commonly believed.

Experimentation

Out-of-door solution cultures

Eureka lemon trees budded on Koethen sweet orange as stocks were obtained from a nursery and were planted bare-root in aerated culture solution in 105-liter capacity galvanized iron containers on February 2, 1938. The containers were coated on the inside with (Gilacoat) asphalt paint and on the outside with aluminum paint.

The culture solution (3) employed had the composition (p.p.m.): Na, 7; K, 185; Ca, 159; Mg, 54; Cl, 10; NO_3, 718; SO_4, 216; PO_4, 105; B, 0.5; Mn, 0.25; and Fe, 0.1. Distilled water was used and iron tartrate was frequently added. The solutions were renewed on April 27, 1938. On June 13, 1938, each culture received 96 p.p.m. Mg and 210 p.p.m. NO_3.

The pH was adjusted by means of dilute solutions of sulphuric or nitric acids, or calcium hydroxide. Two cultures were grown at pH 4.5, using sulphuric acid for adjusting the pH in one case and nitric acid in the other. Calcium hydroxide solution, when required, was used for either culture. Similarly, two other cultures were grown at pH 6.0. A third pair of cultures (49 and 50) received no pH adjustment.

Many leaves curled and fell. On February 14, 1939, the entire tree of culture no. 50 weighed 1256 gm. (2.8 lb.). The control trees were in poor condition (fig. 1). When the pH of such cultures is adjusted to 4.5, recovery is slow because of the loss of leaves and food reserves.

The lemon trees in the pH 6.0 cultures could not be distinguished in their general appearance from the trees in the control cultures. Whenever fruits were produced, they turned yellow at small sizes and were subject to fungus invasion.

TABLE I

pH OF CONTROL LEMON CULTURES AT VARIOUS TIMES

DATE	CONTROL CULTURES; NO ADJUSTMENT OF pH	
	49	50
1938	*pH*	*pH*
April 27	4.5	4.5
May 19	6.0	6.2
June 2	6.0	6.6
June 10	6.4	6.6
June 24	6.7	7.2
July 1	6.6	7.2
July 11	6.6	7.0
July 14	6.6	7.0
July 22	6.8	7.2
July 28	6.8	7.2
August 5	6.8	7.2
August 13	6.8	7.2
September 1	7.0	7.6
September 24	7.2	7.6
October 29	7.6	7.6
November 2	7.3	7.6
November 11	7.3	7.6
November 19	7.3	7.6
November 26	7.4	8.1
December 1	7.4	8.0
December 10	7.4	8.0
December 15	7.8	8.0
December 23	7.6	8.0
1939		
January 3	7.8	8.1
January 19	7.4	8.0
January 27	7.6	8.0
January 30	7.6	8.0

Sulphuric acid was preferred to nitric acid for ease in adjusting and maintaining pH values. The tree growth with sulphuric acid was not inferior to that in which nitric acid was used. The effect of adjusting the pH of two cultures to 4.5 was to bring about excellent growth (fig. 2) when observed on February 14, 1939. The weight of an entire tree grown at pH 4.5 was 3141 gm. (7 lbs.) or about 2.5 times that of the control. The leaves, flowers, and fruits were characteristic of healthy trees. The roots were in excellent condition (fig. 3). One tree from a pH 4.5 culture bore 35 dark green fruits averaging $2\frac{1}{8}'' \times 1\frac{1}{2}''$ while another tree grown at the same pH bore 50 dark green fruits averaging $2\frac{3}{4}'' \times 1\frac{3}{4}''$.

With a similar culture solution made up with tap water, large tanks of 1400-gallon capacity were used in which to grow valencia orange, navel orange, lemon, and grapefruit trees. The pH of the solutions at times increased from 4.5 to above pH 6.0. This was accompanied by marked chlorosis even though iron tartrate was generously supplied. More frequent

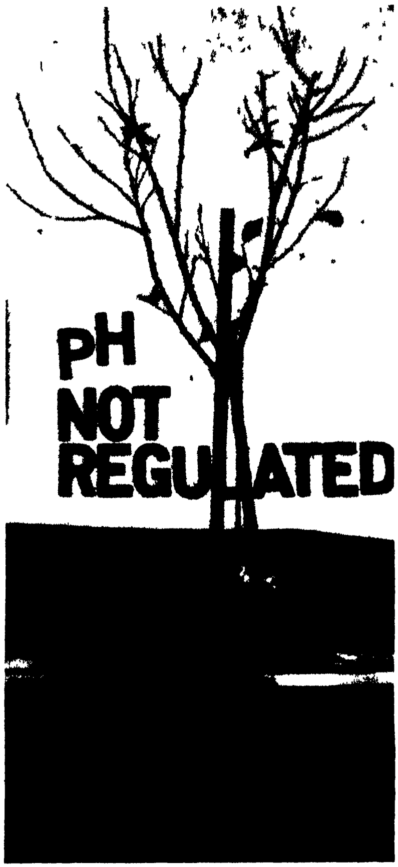


Fig. 1. Eureka lemon tree grown in an aerated culture solution with an initial pH of 4.5 and no subsequent pH adjustment.

adjustment of the pH was necessary to prevent chlorosis. A reduction in the pH of the culture solutions of chlorotic orange trees to pH 4.5 was soon followed by recovery to the green condition, even though many chlorotic leaves fell and weak twigs died. Difficulty is being had with lemon trees in restoring trees grown at or above pH 6.0 to health by reducing the pH to 4.5 because of the weak condition of the trees and loss of leaves.

Solution cultures in the glasshouse

Valencia orange cuttings.—Experiments were begun March 15, 1937, with valencia orange cuttings in 12-gallon crocks of aerated culture solution made with distilled water. The basis of the culture solution was (p.p.m.): Na, 3.5; K, 71; Mg, 27; Cl, 5; SO_4, 196; B, 0.5; Mn, 0.25; Zn, 0.1; and Al (as aluminum citrate), 7.0. Every 18 liters of solution received 6.2

FIG. 2. Eureka lemon tree treated in every way like the tree shown in figure 1 except that the pH was adjusted to 4.5.

gm. calcium sulphate ($CaSO_4$ $2H_2O$) or 2520 ml. of solution of distilled water saturated with calcium sulphate. Small additions of iron tartrate were made from time to time.

Each set of cultures consisted of 4 crocks Two crocks received the phosphate only for a short period before the solutions were renewed, while the other 2 crocks received the phosphate at every solution renewal. The use of phosphate (KH_2PO_4) involved the addition of 52.5 p.p.m. PO_4 and 21 p.p.m. of K. The solution in one of each pair of crocks was adjusted to pH 4.5 and the others were adjusted to pH 6 0. Adjustments of pH were made twice every week with sulphuric acid or calcium hydroxide solutions.

Each set of 4 crocks received equal concentrations of nitrogen (122 p.p.m. N or 539 p.p.m. NO_3). The source of nitrogen was different in each set of cultures.

Every culture contained 6 valencia orange cuttings at the start. Replacements were very numerous in the cultures with phosphate continually present and in some of the cultures at pH 6.0 even with phosphate supplied at intervals.

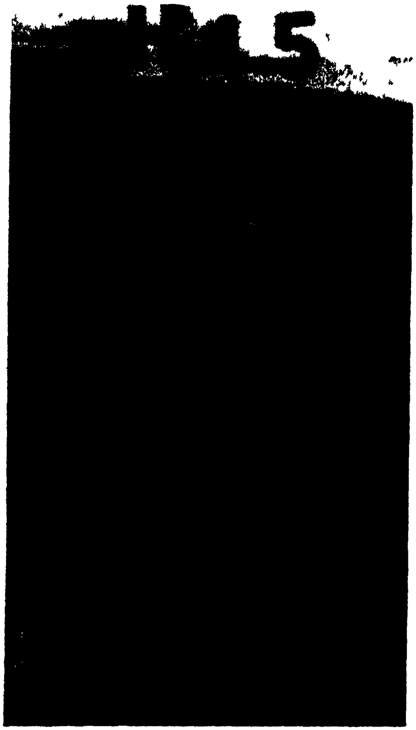


FIG. 3. Root system of Eureka lemon tree shown in figure 2.

Table II presents data of interest in connection with young plants. When phosphate was continuously supplied, the young cuttings flowered and fruited heavily, but were unable to set any of the fruit, presumably on account of the low carbohydrate reserves. There was little or no vegetative growth as a result of this excessive fruiting. In other cultures in which large budded trees were used in the glasshouse (as with the lemon tree experiment previously described in this paper), phosphate continually present in the solution does not prevent excellent growth once the plants have reached an age suitable for bearing and maturing fruit. When of bearing age, it is advantageous to have phosphate present at all times.

So many replacements had to be made in some cases (table II) that either the cultures were abandoned or they were finally supplied with large cuttings grown in the same solution at pH 4.5 in which phosphate was present only at intervals. Even then the subsequent growth in many cases was poor. All of the trees were photographed on February 14, 1939, using approximately the same focal distance. In May, 1939, the same growth relationship held as on February 14 except on a higher level.

TABLE II

REPLACEMENT OF VALENCIA ORANGE CUTTINGS GROWN IN CULTURE SOLUTIONS

CROCK NO.	SOURCE OF NITROGEN	pH	PO_4 ALWAYS	REPLACED CUTTINGS	REMARKS ABOUT PLANTS IN CULTURES
					May 24, 1939
49	Calcium nitrate	4.5	No	0	Green
50	" "	6.0	No	10	Yellow
51	" "	4.5	Yes	9	Very poor, near dead
52	" "	6.0	Yes	7	
53	Ammonium nitrate	4.5	No	1	Green
54	" "	6.0	No	6	Green
55	" "	4.5	Yes	19	All dead
56	" "	6.0	Yes	6	Small and very yellow
29	Ammonium chloride	4.5	No	1	Green
30	" "	6.0	No	6	Yellow-green
31	" "	4.5	Yes	9	Small and yellow
32	" "	6.0	Yes	17	
33	Ammonium sulphate	4.5	No	2	Yellow-green
34	" "	6.0	No	8	
35	" "	4.5	Yes	26	Small
36	" "	6.0	Yes	20	
37	Sodium nitrate	4.5	No	1	Leaf burn at times
38	" "	6.0	No	16	All dead
39	" "	4.5	Yes	9	Very poor
40	" "	6.0	Yes	9	All dead
41	Magnesium nitrate	4.5	No	2	Yellow-green
42	" "	6.0	No	12	All dead
43	" "	4.5	Yes	6	
44	" "	6.0	Yes	9	
45	Potassium nitrate	4.5	No	4	Yellow-green
46	" "	6.0	No	6	
47	" "	4.5	Yes	11	All dead
48	" "	6.0	Yes	8	

Figure 4 shows the comparative growth of cultures 49 and 50, (see table II) in which calcium nitrate was the source of nitrogen. The best growth was made at pH 4.5, in a culture in which phosphate was present only at intervals. At pH 6.0, the leaves were quite yellow. Tests of the solutions were occasionally made to ascertain whether the nitrate was greatly reduced.

Table III shows the pH values of the calcium nitrate culture solution (crock 49) whenever regulation to pH 4.5 was made. Observation of the growth made it appear that the shift toward higher pH values than 4.5 were largely made during the production of new leaves and that the low pH values occurred principally as the foliage was maturing. HAAS and HALMA (5) have shown that maturing citrus leaves contain increasingly large amounts of calcium. If the nitrate and other anions were not ab-

FIG. 4. Calcium nitrate as a source of nitrogen for valencia orange cuttings grown in culture solutions with phosphate not continuously supplied. Left, pH 6.0; right, pH 4.5.

TABLE III

pH OF THE CULTURE SOLUTION BEFORE READJUSTMENT TO A GIVEN pH; PHOSPHATE IN CULTURES AT INTERVALS

Date	Calcium nitrate culture 49, pH 4.5	Ammonium nitrate culture 53, pH 4.5	Date	Calcium nitrate culture 49, pH 4.5	Ammonium nitrate culture 53, pH 4.5	Ammonium nitrate culture 54, pH 6.0
1938	*pH*	*pH*	*1939*	*pH*	*pH*	*pH*
June 7	7.5	5.9	Jan. 11	7.0	4.5	
11	7.0	6.0	13	7.0	4.5	
14	7.1	5.0	17	7.0	3.0	
17	6.7	4.5	20	7.0	3.0	
21	6.8	4.0	24	6.0		
24	6.6	4.5	31	6.8	4.5	
28	6.0	4.0	Feb. 3	6.5	4.5	
July 5	4.5	3.8	7	5.0	4.5	
8	3.8	3.8	10	5.0	4.5	
22	5.7	4.3	15	4.5	4.5	
Aug. 3	4.5	3.0	17	4.5		
9	4.5	3.0	21	4.0	4.5	
12	4.2	4.0	24	4.0	4.2	
16	4.1	4.0	28	3.8	4.0	
19	4.1	3.8	Mar. 3	4.3	4.3	
23	4.0	3.8	7	4.0	3.8	
30	4.3	3.8	10	4.0	3.8	
Sept. 2	4.5	4.5	14	4.1	4.1	5.0
6	4.2	4.2	17	4.0	4.2	4.2
8	4.3		22		4.5	6.0
13	4.5	4.5	24	5.0	4.3	4.4
16	4.2	4.2	27	5.0	4.0	4.0
21	4.2	4.2	31	5.0	3.8	6.0
23	4.3		Apr. 4	4.5	4.0	6.0
Oct. 4	4.5	6.0	8	4.5		6.0
Nov. 1	6.0	3.8	12	4.2	4.0	6.0
8	4.2	4.5	14	4.2	4.0	6.0
15	4.5	4.5	18	4.3	4.2	6.0
18	4.3	4.2	21	4.2	5.5	4.5
23	4.5	4.3	25	4.0	4.8	4.4
25	4.2	4.3	28	4.1	4.2	4.2
28	4.3	4.3	May 2	4.0	4.2	4.2
Dec. 6	4.3	4.5	5	4.5	4.5	4.4
9	4.2	4.2	10	4.2	4.4	4.2
13	4.2	4.2	13	4.2	4.4	4.2
16	4.5	4.2	17	4.4	5.0	4.2
20	4.2	4.2	19	4.5	5.8	6.0
23	4.5	4.3	23	4.0	4.5	4.5

sorbed at the same rate as the calcium, the tendency would be to increase the acidity of the culture solution during the periods of heavy calcium intake.

The calcium nitrate culture no. 50 (adjusted to pH 6.0) was relatively easy to keep at that value, the tendency largely being to shift to slightly higher pH values. The effect of the higher pH (6.0) of the culture solution on the growth of the cuttings is very marked (fig. 4).

Figure 5 shows valencia orange cuttings grown in culture solutions adjusted to pH 4.5 and 6.0 when ammonium nitrate was the source of nitrogen and when phosphate was not continuously present in the solution. Growth at pH 4.5 was approximately the equal to that when calcium nitrate was used (fig. 4). At pH 6.0, the growth was greater and of better color than

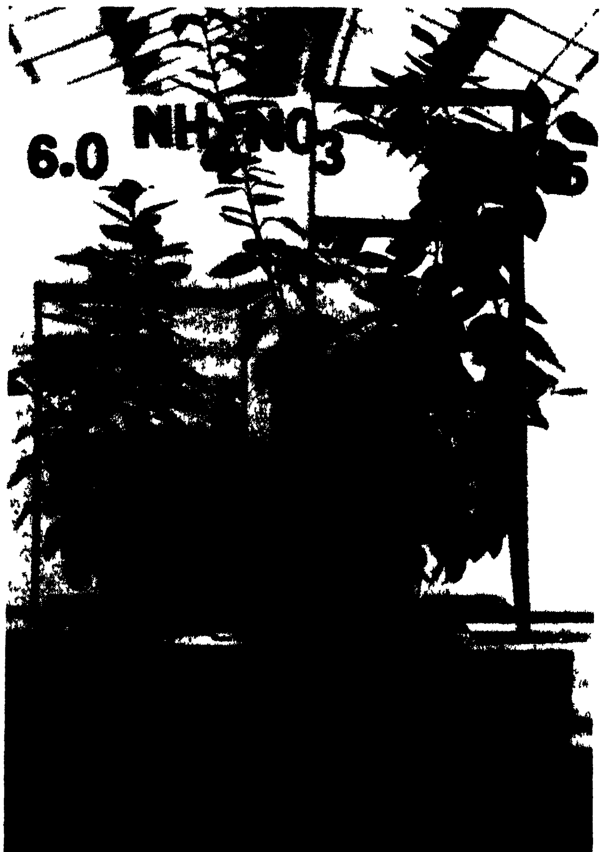


FIG. 5. Ammonium nitrate as a source of nitrogen for valencia orange cuttings grown in culture solutions with phosphate not continuously supplied. Left, pH 6.0; right, pH 4.5.

that produced with calcium nitrate. Table III shows some of the pH values reached by the solutions before readjustments of pH were made. The calcium nitrate culture solution either remained at pH 6.0 or went slightly higher while the tendency for the ammonium nitrate culture solution, adjusted to pH 6.0, was to shift toward greater acidity. This gave the am-

monium nitrate culture an advantage over the calcium nitrate culture in making the greater and healthier growth in the culture intended to be held at pH 6.0. Despite the difficulties of pH maintenance, figure 5 shows that at pH 6.0 the ammonium nitrate culture made fairly good growth but not as much as at pH 4.5. At these same pH values with the phosphate continuously present, young cuttings did poorly as seen in table II.

Figure 6 shows the growth of cultures in which ammonium chloride or

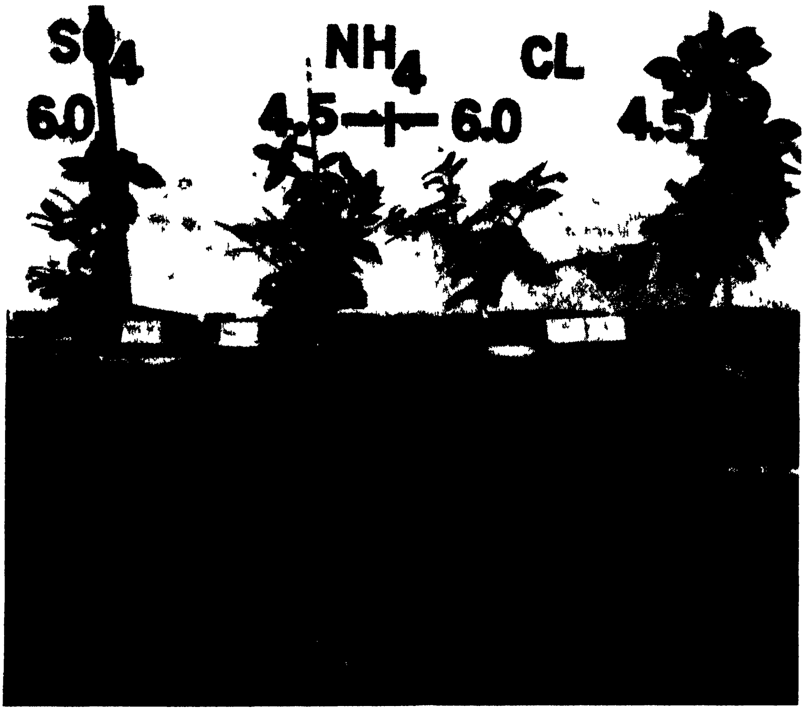


FIG. 6. Growth of valencia orange cuttings in solution cultures with ammonium sulphate or chloride as the source of nitrogen and with phosphate not continuously supplied. Right to left: ammonium chloride, pH 4.5 and 6.0; ammonium sulphate, pH 4.5 and 6.0.

sulphate was the source of nitrogen and in which phosphate was supplied at intervals. The plants at pH 4.5 did not make nearly as good growth as the ammonium nitrate or calcium nitrate cultures. The cuttings at pH 6.0 grew so poorly that cuttings were first grown in similar solutions at pH 4.5 and were then transferred to the pH 6.0 cultures.

Although the solutions at pH 4.5 tended toward lower pH values, the increasing acidity is not considered to be responsible for the smaller growth (fig. 6). It is more than likely that the enforced absorption of

ammonium as the sole source of nitrogen is injurious to the health of the plant. It is to be noted that no solution culture formula that makes use of ammonium nitrogen excludes the use of nitrate but rather makes the nitrate nitrogen predominantly greater than the ammonium nitrogen. Investigators admit using ammonium salts in small amounts in their culture solutions for the primary purpose of acidification or ease of pH maintenance.

It is desirable to interrupt the discussion at this point in order to report an experiment on the effect of increasing the ammonium nitrogen while keeping the total nitrogen constant. A series of three 48-liter crocks were used for aerated solution cultures in each of which three Eureka lemon cuttings were placed. The cultures have been growing more than a year, during which time the pH of the solutions were adjusted to 4.5. With increasing concentrations of ammonium sulphate there is greater difficulty in maintaining the pH of the culture solution at 4.5. The culture solution used in each crock had the following composition (p.p.m.): Na, 7; K, 185; Mg, 54; Cl, 10; NO_3, 225; SO_4, 216; PO_4, 105; B, 0.5; Mn, 0.25; and Fe, 0.1. Iron tartrate was supplied at intervals and distilled water was used throughout the experiment. No zinc or aluminum was used. To these culture solutions were added calcium nitrate and ammonium sulphate solutions as shown in table IV.

TABLE IV

SOURCE AND CONCENTRATION (P.P.M.) OF NITROGEN ADDED TO EUREKA LEMON SOLUTION CULTURES

	CALCIUM	NITROGEN (ADDED AS CALCIUM NITRATE)	SULPHATE	NITROGEN (ADDED AS AMMONIUM SULPHATE)
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Crock 26	298	209	48	14
Crock 27	279	195	95	28
Crock 28	238	167	190	56

Figure 7 shows the effect of increasing the ammonium nitrogen from 14 to 28 to 56 p.p.m., respectively, while adding sufficient nitrate to make the total nitrogen 223 p.p.m. As the ammonium nitrogen increased, the growth decreased. The total fresh weights of the trees and fruits were: 2444, 1758, and 1516 grams respectively, and the number of fruits were 12, 13, and 9, respectively, with increasing ammonium nitrogen. The enforced absorption of ammonium in the absence of sufficient nitrate does not appear to be beneficial in the growth of lemon cuttings.

Resuming our consideration of various sources of nitrogen at pH 4.5 and 6.0 with valencia orange cuttings continuously supplied with phosphate or supplied only at intervals, the study of sodium, potassium, and magnesium

FIG. 7. Effect (in solution cultures of Eureka lemon cuttings) of increasing the ammonium nitrogen in the presence of a constant amount of total nitrogen. The pH was adjusted to 4.5. As the ammonium nitrogen was increased, growth decreased. Left to right: 14, 28, and 56 p.p.m. nitrogen.

nitrates as sources of nitrogen should be of interest. When these nitrates were used the growth of the cuttings was markedly inferior to that obtained when calcium or ammonium nitrate was used (fig. 8). Better growth was made at pH 4.5 than at 6.0 and when PO_4 was supplied at intervals rather than when continuously present. The leaves of the sodium nitrate culture burned upon becoming mature and upon becoming affected by accumulations of certain constituents in the leaves. The root systems were extremely long, with a scarcity of laterals.

The effect of pH on large-size valencia orange cuttings grown with phosphate continuously present was next studied because young citrus cuttings do not succeed very well when phosphate, in the concentration used,

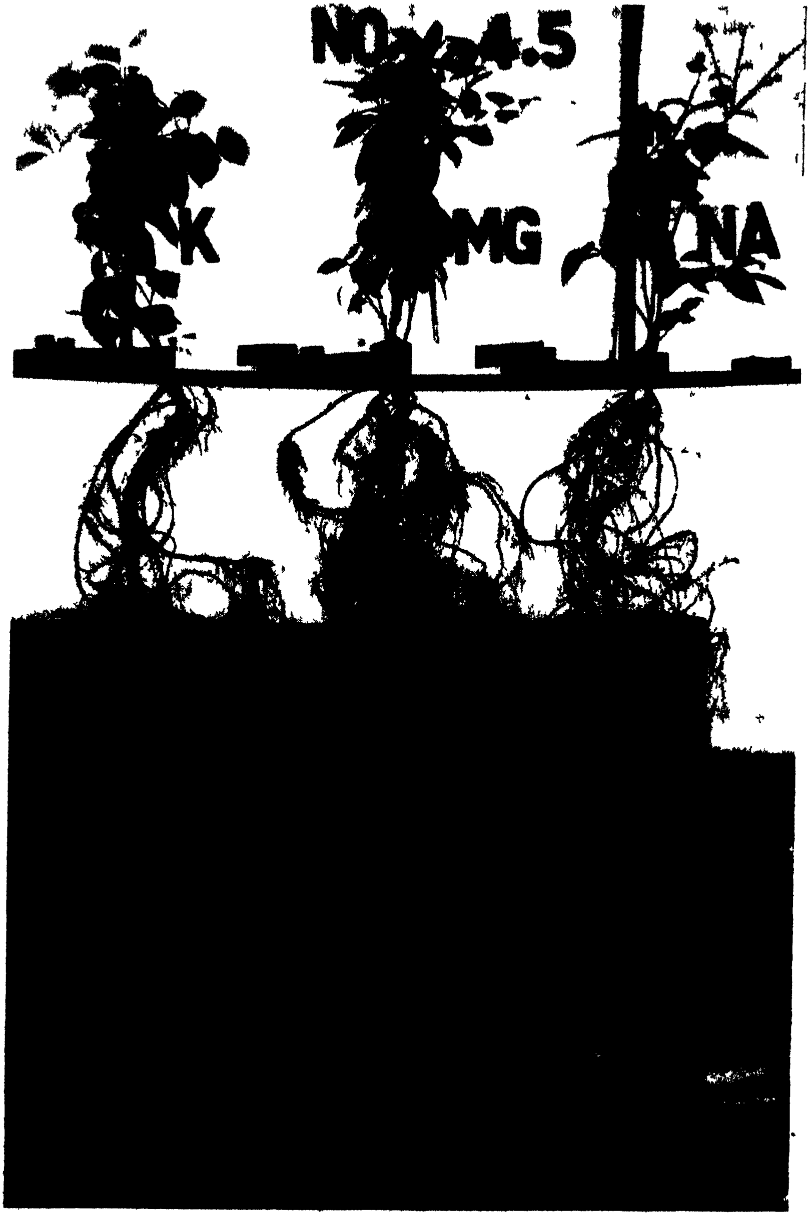


FIG. 8. Growth of valencia orange cuttings in culture solutions adjusted to pH 4.5 and with phosphate not continuously supplied. The sources of nitrogen, left to right: potassium, magnesium, and sodium nitrate.

is present continually in the culture solution. The young rooted cuttings were first grown in the same kind of solutions as later, except that in the young stages of growth the phosphate was present in the culture solution only at intervals and the pH was adjusted to 4.5. When a year or more in age, the cuttings were then supplied continuously with phosphate and the pH values of the solution were adjusted to 4.5 and 6.0 respectively. The largest plants purposely were used for the cultures later to be maintained at pH 6.0 in order to give them a good chance to make excellent growth.

It was found that at pH 6.0 with phosphate continuously present in the culture solution, the leaves were chlorotic while at pH 4.5 the leaves were green. The roots of the plants at pH 4.5 were considerably larger than at 6.0 despite the fact that when the differential pH values were begun, the plants used for the pH 6.0 cultures were superior to those used for the cultures at pH 4.5.

SOIL CULTURES IN GLASSHOUSE

LEMON CUTTINGS IN PH ADJUSTED SOIL CULTURES.—River soil obtained near Riverside was used in 5-gallon tin containers coated with asphalt paint. One-half pound of dried blood was mixed thoroughly with the soil in each container. Small known aliquots of this soil were used to determine the amount of dilute sulphuric acid or sodium hydroxide necessary to add to the soil in order to secure various initial values when a ratio of 1 part of dry soil to 5 of distilled water was used in obtaining the soil extract. The pH of this soil suspension was determined with a glass electrode.

On December 27, 1938, the experiment was begun with 3 Lisbon lemon cuttings in the soils adjusted to various pH values. The cuttings at the lowest pH value had made one cycle of growth and part of the second cycle while the cuttings at the next higher pH value were just starting growth and none had started at the higher pH values. On February 20, 1939, the Lisbon lemon cuttings were discarded and 3 Eureka lemon cuttings were planted in each soil culture. By May 10, 1939, the culture that was begun at the lowest pH value had made the most growth; the culture begun at the second lowest pH value ranked second in growth, while in cultures begun at higher pH values, only 1 cutting put out a small shoot. The cuttings at the high pH levels failed to grow even though replants were used repeatedly. When such injured cuttings were planted in more acid soil, many recovered.

When these soils were first adjusted to definite pH values on a 1:5 soil-water ratio basis, the pH values of samples taken at the start of the experiment were: 4.18, 5.90, 8.05, 8.89, and 9.97, respectively. Determinations of pH by the 1:5 soil-water ratio method were made on soil samples taken and dried on May 2, 1939. The pH values were: 4.96, 5.60, 7.25, 7.27, and 7.81 respectively. Such changes in the pH of adjusted soils are common.

SHEAR (7) recognized this fact and waited 2 years for soils to attain their equilibrium. When the pH of the soil samples of May 2, 1939 was determined with the spear-type glass electrode (6), the values obtained were: 4.74, 4.93, 6.48, 6.62, and 6.74 respectively at moisture contents of 18.1, 18.0, 17.1, 13.0, and 21.1 per cent. respectively (dry soil basis). Growth at the lowest pH values was better than at the high pH values.

Before experiments with various sources of nitrogen in soil are discussed, reference should be made to the results (4) obtained with avocado seedlings grown in Hanford soil in asphalt-coated containers in the plant pathology temperature tanks maintained at 10°, 17°, and 24° C. respectively. In these temperature tanks the soil temperature is controlled while the temperature of the tops of the trees approximates that of the glasshouse. Two containers at each soil temperature received a calcium nitrate solution while two other containers at the same temperature received an ammonium sulphate solution. All containers thus received at the beginning of the experiment, equal amounts of nitrogen, either in the form of calcium nitrate or ammonium sulphate. Distilled water was used at all times. At the conclusion of the experiment, soil samples were taken from the containers and dried. pH determinations of water suspensions (1:5 soil-water ratio) were made and the results shown in table V were obtained.

TABLE V

EFFECT OF SOIL TEMPERATURE AND SOURCE OF NITROGEN ON THE pH OF (1: 5 SOIL-WATER RATIO) SUSPENSIONS

SOIL TEMPERATURE	FERTILIZER USED (EQUIVALENT NITROGEN)			
	CULTURE NUMBER	CALCIUM NITRATE	CULTURE NUMBER	AMMONIUM SULPHATE
°C.		pH		pH
10	1	7.49	3	5.80
	2	7.48	4	5.92
17	5	7.49	7	5.61
	6	7.43	8	5.67
24	9	7.27	11	5.53
	10	7.49	12	5.49

The pH of the calcium nitrate-treated soil remained approximately the same at the various soil temperatures while that of the ammonium sulphate-treated soil was progressively lower with increasing soil temperature. Undoubtedly temperature affects the rate of nitrification of ammonium-nitrogen and the production of acid which lowers the pH. This fact should be

considered in growth studies that involve the use of ammonium sulphate at various soil temperatures.

EFFECT OF NITROGEN SOURCE ON GROWTH OF LEMON CUTTINGS.—In an experiment with equivalent amounts of nitrogen from various sources, small tin containers (6 inches in diameter × 6¾ inches high) coated with asphalt paint were used with about 3650 grams of air-dry pasture soil (Hanford series). 0.1825 gram of nitrogen was used to give a concentration of 50 p.p.m. of nitrogen (air-dry basis). A Lisbon lemon cutting was planted in each container on January 29, 1937. Distilled water was used at all times and the containers were without drainage. On February 25, 1937, the source of nitrogen had shown an effect (table VI) on the occurrence of new growth.

TABLE VI

SOURCE OF NITROGEN AND OCCURRENCE OF NEW GROWTH

SOIL TREATMENT	NUMBER OF CULTURES USED	CUTTINGS WITH NEW GROWTH
Control	9	0
Potassium nitrate	8	0
Calcium nitrate	8	1
Sodium nitrate	8	4
Magnesium nitrate	8	5
Ammonium nitrate	8	5
Ammonium chloride	8	4
Ammonium sulphate	8	8

The addition of certain forms of nitrogen to the soil caused a response in growth more quickly than when other forms of nitrogen were used. Potassium and calcium nitrate were the slowest in initiating new growth while ammonium sulphate was the most rapid. On March 31, 1937, the leaves of the ammonium sulphate-treated cuttings were the darkest green of all the cultures and had started their second cycle of growth before the others. On April 12, 1937, the ammonium sulphate cultures showed the most rapid growth. The sodium nitrate cultures were perhaps equivalent to the ammonium chloride cultures, the sodium and especially the chlorine being unfavorable. The slower action of nitrate nitrogen as compared with ammonium nitrogen is attributed by some investigators to the necessity of the plant first having to reduce the nitrate before such nitrogen is available for protein synthesis. In soil, it appears from the results obtained that the pH is a factor worthy of consideration in this difference in the rate of growth when nitrate or ammonium nitrogen is used.

The experiment was continued and on June 21, 1937, 50 p.p.m. of PO_4 as $Ca(H_2PO_4)_2 \cdot H_2O$, or 0.1825 grams P, was added to half of the cultures. On July 10, 1937, the phosphate treatment was repeated without any response being noted at any subsequent time.

The experiment was continued until December 14, 1937. Soil samples were taken and dried before terminating the cultures, the soil cores being taken the full depth of the containers. The pH values of (1:5 soil-water ratio) suspensions of these samples were determined with the glass electrode and the results are shown in table VII.

TABLE VII

EFFECT OF SOIL TREATMENT AND THE GROWTH OF LEMON CUTTINGS ON SOIL pH

FERTILIZER USED (EQUIVALENT NITROGEN)	pH OF (1: 5 SOIL-WATER RATIO) SUSPENSION	
	PHOSPHATE FERTILIZATION	NO PHOSPHATE FERTILIZATION
	pH	*pH*
Control	6.58	6.61
Ammonium sulphate	5.92	5.95
Calcium nitrate	6.28	6.27
Potassium nitrate	6.37	6.18
Magnesium nitrate	6.30	6.42
Ammonium chloride	5.82	5.87
Ammonium nitrate	6.13	6.33
Sodium nitrate	6.49	6.58

Of the plants in the two most acid soils, the cuttings in ammonium-sulphate-treated soil were healthy while those in the ammonium-chloride-treated soil were injured by the chlorine. The cuttings in the ammonium-sulphate-treated soil made the most rapid and the healthiest growth of any of the nitrogen treatments.

INJURY AND RECOVERY OF LEMON CUTTINGS IN SOIL WITH VARIOUS SOURCES OF NITROGEN.—An experiment was conducted with lemon cuttings in containers of soil similar to those used previously. Each container was given 3844 gm. of air-dry soil which was a screened and well mixed lot of pasture soil of the Hanford series. A solution containing 0.1825 gm. of nitrogen was added to each culture (about 48 p.p.m. of nitrogen). Distilled water was used at all times.[1] The experiment was begun on April 20, 1937. The following sources of nitrogen were used: ammonium sulphate; ammonium sulphate commercial; calcium nitrate; calcium nitrate commercial; the nitrates of potassium, magnesium, sodium and ammonium; ammonium chloride; ammonium citrate; ammonium hydroxide; diammonium monohydrogen phosphate; and ammonium sulphate (in combination with 0.4 gram potassium sulphate per culture). An additional fertilizer application was made on July 9, 1937; on December 15, 1937, 2.5 times the usual application was made.

[1] Unless otherwise specified, C. P. chemicals, equivalent nitrogen, and distilled water were used and no drainage was provided. The cultures were protected at all times from rain damage.

In December, 1937, some of the cultures were given various amounts of N/10 sulphuric acid ranging from 1000 to 2500 ml. In a very short time practically all of these cultures were badly injured near the place of contact of the acid with the trunk and either died or were discarded before death. In such small containers it was not feasible to keep the strong acid from contacting and injuring the trunk.

On April 30, 1938, the remaining cultures differed in appearance, depending upon the source of nitrogen. Some cultures were declining while others, ammonium sulphate for example, were consistently healthy.

The cultures, except those treated with ammonium sulphate, were divided into certain groups for further treatment: 1. Cultures untreated, control. 2. Cultures watered with N/1000 sulphuric acid whenever water was required. 3. Cultures each received 2.5 grams of sulphur. 4. Cultures each received 5 grams of sulphur.

On August 10, 1938, pH determinations with the glass electrode were made by the 1:5 soil-water ratio method. Table VIII gives results ob-

TABLE VIII

EFFECT OF NITROGEN FERTILIZATION AND ACID OR SULPHUR TREATMENT ON THE pH OF (1:5 SOIL-WATER RATIO) SUSPENSONS

FERTILIZER TREATMENT (EQUIVALENT NITROGEN)	TREATMENT ON APRIL 30, 1938			
	NONE	2.5 GRAMS SULPHUR	5 GRAMS SULPHUR	N/1000 SULPHURIC ACID CONTINUALLY
	pH	*pH*	*pH*	*pH*
Control	7.01		6.47	
Ammonium sulphate	4.29–4.91			
Ammonium sulphate, commercial	4.35–4.70			
Calcium nitrate	7.30	4.70	6.50	7.57
Calcium nitrate, commercial	7.06	5.48	5.10	7.23
Potassium nitrate	7.23–7.60	5.79–6.20	6.07	7.37
Magnesium nitrate	6.94–6.95	6.42	6.11	7.52
Sodium nitrate	8.37	5.29–6.00	5.90	8.16
Ammonium chloride	4.71		4.93	5.05
Ammonium nitrate	5.53	4.13	4.49	6.10
Ammonium citrate	6.09–6.41	4.81	4.01	6.74
Ammonium hydrate	6.10–6.41	4.35		6.40
Acid ammonium phosphate	5.44	4.50	4.42	5.43
Ammonium sulphate and potassium sulphate	4.12–4.67			

tained. The ammonium-nitrogen fertilizers generally made the soil more acid while the tendency of the nitrate-nitrogen fertilizers was to make the soils slightly more alkaline. The larger the growth of the plant, the more distilled water (pH 5.2) was used. This was an aid in preventing a large increase in the pH of the soil. The use of very dilute sulphuric acid appears not to have reduced the pH of the soil as determined by the methods

TABLE IX

FRESH AND DRY WEIGHTS OF LEAVES, TWIGS, AND ROOTS OF LEMON CUTTINGS AS AFFECTED BY SOURCE OF NITROGEN AND pH OF THE SOIL MEDIUM

Fertilizer treatment (equivalent nitrogen)	No treatment						2.5 grams sulphur					
	Leaves		Twigs		Roots		Leaves		Twigs		Roots	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	8.8	2.5	8.5	3.9	33.1	8.8	7.0	2.1	9.0	4.1	37.5	10.0
Ammonium sulphate	278.0	91.7	505.0	272.0	490.5	189.0	...	...	...	...	...	...
Ammonium sulphate, commercial	251.5	81.7	448.0	242.0	566.0	237.0	...			...	...	...
Calcium nitrate	15.0	4.0	17.0	8.0	25.3	9.0	60.2	19.0	40.7	16.5	51.5	15.0
Calcium nitrate, commercial	23.3	7.0	30.0	14.9	31.0	10.2	52.0	17.0	66.0	34.0	66.0	24.4
Potassium nitrate	26.5	7.0	34.5	15.0	36.0	11.4	61.0	18.0	65.5	31.8	69.7	23.9
Magnesium nitrate	18.3	5.3	21.7	10.2	30.5	9.3	54.0	17.7	39.0	19.1	60.2	18.0
Sodium nitrate	26.2	7.0	32.8	13.1	30.0	8.2	70.2	21.9	46.5	21.4	62.0	21.0
Ammonium chloride	4.0	1.3	4.9	3.0		...				...	...	...
Ammonium nitrate	21.5	6.0	25.1	11.0	35.4	10.0	50.0	16.0	34.0	15.3	53.0	16.6
Ammonium citrate	23.8	7.0	31.8	14.7	42.5	13.2	38.8	12.0	38.8	19.2	57.0	21.0
Ammonium hydroxide	25.5	8.0	27.0	13.7	35.7	11.1	37.8	12.0	47.5	23.0	68.0	23.9
Acid ammonium phosphate	18.7	5.4	28.4	15.0	41.7	13.2	56.3	18.5	50.2	23.5	65.7	22.0
Ammonium sulphate and potassium sulphate	255.5	80.4	448.3	238.2	516.5	207.0		...		...	...	...

TABLE IX.—(*Continued*)

Fertilizer treatment (equivalent nitrogen)	5 grams sulphur						N/1000 sulphuric acid continually					
	Leaves		Twigs		Roots		Leaves		Twigs		Roots	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Control	7.5	2.5	10.0	4.7	29.8	8.0	7.0	2.0	9.0	4.0	31.7	8.2
Ammonium sulphate												
Ammonium sulphate, commercial												
Calcium nitrate	60.1	18.5	45.3	22.0	77.4	24.8	61.8	19.8	33.1	15.0	55.2	16.5
Calcium nitrate, commercial	72.0	23.0	70.0	34.4	75.5	26.1	54.7	19.0	52.1	25.4	64.7	20.5
Potassium nitrate	57.9	17.7	62.8	28.7	77.2	25.9	64.8	18.7	48.1	21.1	70.5	20.4
Magnesium nitrate	48.5	16.1	39.5	19.7	70.1	23.2	51.3	18.0	42.3	21.1	66.1	23.0
Sodium nitrate	55.0	16.9	46.0	21.9	56.5	18.2	49.2	15.0	40.3	18.1	48.0	15.0
Ammonium chloride	4.8	1.3	3.7	1.8	4.2	1.2	9.1	2.1	6.8	4.0	13.8	4.0
Ammonium nitrate	60.5	19.0	37.2	17.7	60.0	19.2	49.5	17.6	40.3	19.9	70.1	22.1
Ammonium citrate	28.7	8.2	39.2	19.2	63.0	18.5	39.4	12.6	45.5	23.0	71.1	22.0
Ammonium hydroxide							38.7	12.7	46.5	23.0	69.0	23.1
Acid ammonium phosphate	48.9	16.9	56.0	27.2	71.0	25.0	67.1	21.7	61.5	29.0	83.3	28.0
Ammonium sulphate and potassium sulphate												

used. In sampling each soil culture, however, a composite or average was made of several cores taken to the bottom of the container and the pH of the upper soil may have been lower than that of the deeper soil. This at least is the condition met with commonly in the field.

When attempts were made to lower the pH of the entire mass of soil by the use of N/10 sulphuric acid, the concentration of acid used proved injurious when in contact with the trunk in these small containers. Attempts were made to ridge the soil about the tree and prevent the contact but it was not feasible in the small size container.

When sulphur was used instead of N/10 sulphuric acid, no difficulties resulted from concentrations of acid against the trunk. The formation of acidity is gradual enough and moves gradually downward, with no apparent result except in the ease of water penetration. There is considerable evidence in the marked response of the cuttings to the use of sulphur or dilute sulphuric acid to indicate that lemon cuttings grow better under more acid soil conditions than is usually considered most favorable.

The data in table IX gives the weights of the lemon cuttings in the various cultures at the end of the experiment. The cultures were photographed August 29, 1939, and show the effectiveness of the acidifying treatments of April 30, 1938. Figure 9 is typical of the results obtained

FIG. 9. Lemon cuttings grown in Hanford soil that received calcium or potassium nitrates as the source of nitrogen. Injury resulted but recovery was brought about by the use of sulphur or sulphuric acid.

and shows the marked benefit to lemon cuttings in nitrogen-fertilized Hanford soil in which the soil pH is taken into consideration. When nitrogen was not supplied to the cultures, acid treatment gave very little improvement because of the limitations on growth. These studies have thrown light on one phase of the mode of action of copper sulphate applications in soil upon the growth response by citrus cuttings.

COPPER SULPHATE EFFECTIVENESS IN RELATION TO pH IN LEMON CULTURES. —An experiment was begun with undrained containers in which was placed 4000 gm. (air-dry basis) of Hanford soil from the same lot previously used. A solution of copper sulphate was added to the soil cultures as shown in table X. The soil in each container was thoroughly mixed after the addition of copper sulphate. On March 8, 1938, a Eureka lemon cutting was planted in each container of soil. 0.1825 gm. of nitrogen in solution was added to each culture. Distilled water was used at all times.

Ammonium sulphate was used as the source of nitrogen in one series of cultures while calcium nitrate was used as the source of nitrogen in the other series. 0.3650 gm. of nitrogen was added August 12, 1938, and again on December 19, 1938, to the cultures of each series.

In January, 1939, the leaves of the calcium-nitrate series were turning yellowish green and were being lost while in the ammonium-sulphate series the leaves were dark green and securely attached. On April 5, 1939, the weights of the cuttings were determined (table X). Composite cores of soil were taken at that time from each culture to the full depth of the container for pH determination with the spear-type glass electrode.

The calcium-nitrate cultures had 60 immature leaves of 48 gm. fresh weight while the ammonium sulphate cultures had 286 immature leaves of 241 gm. fresh weight and in addition 4 lemon fruits $\frac{1}{4}''$–$1''$ in size.

Table X shows that as the copper sulphate concentration was increased in the calcium nitrate series, fewer leaves were lost. High concentrations of copper depressed growth. There was little if any benefit from the use of copper sulphate in the ammonium-sulphate series, but the benefit in the calcium-nitrate series was pronounced.

Table XI reveals that the use of copper sulphate in most cases assisted in lowering the pH of the soil in the calcium-nitrate series. The pH determinations were made on fresh composites at the soil moisture content and on the dried soil by the 1:5 soil-water ratio method. Table XI shows that when the pH values were lowered sufficiently, the cuttings appeared healthy. These results were obtained with the same lot of soil in which acidification by means of sulphur or sulphuric acid also was beneficial. If copper is deficient in cuttings in the calcium-nitrate series it must be the result of high soil pH because sulphur, sulphuric acid, or ammonium sulphate corrected the difficulty without the addition of copper. In ex-

TABLE X

GROWTH OF EUREKA LEMON CUTTINGS AS AFFECTED BY VARIOUS CONCENTRATIONS OF COPPER SULPHATE WITH EQUIVALENT NITROGEN FROM CALCIUM NITRATE OR AMMONIUM SULPHATE SOURCE

Copper added to air-dry soil	Mature leaves						Twigs				Roots			
	Calcium nitrate			Ammonium sulphate			Calcium nitrate		Ammonium sulphate		Calcium nitrate		Ammonium sulphate	
	No. of leaves	Fresh wt.	Dry wt.	No. of leaves	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
p.p.m.		gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0.5	0			79	47	15.5	14	5.7	45	21.1	10	3.0	74	23.7
1.0	0			73	64	21.5	8	3.3	57	30.2	10	2.7	68	23.5
2.5	6	5.0		64	52	17.0	11	5.0	50	23.9	15	4.3	74	20.7
5.0	2			82	53	17.8	14	6.5	52	23.7	17	5.3	65	20.0
7.5	16	18.0	5.0	86	47	15.9	32	15.1	32	15.4	48	15.0	54	18.0
10.0	18	13.0	4.9	79	48	17.4	22	9.8	52	26.5	25	7.8	67	24.9
15.0	41	25.0	9.0	89	53	18.1	18	8.3	42	22.6	26	8.0	61	23.3
20.0	50	36.0	12.1	81	45	15.5	33	15.0	30	13.9	47	18.1	50	15.7
30.0	29	19.5	6.5	53	45	14.8	18	7.5	47	23.7	19	4.5	69	26.9
40.0	60	44.0	15.3	86	49	15.8	45	21.3	38	20.0	82	32.7	48	18.0
50.0	53	42.0	13.7	74	40	14.7	30	14.0	46	21.1	41	12.7	76	22.7
75.0	23	12.0	4.0	64	36	11.9	8	4.0	38	17.8	13	4.0	60	23.9
100.0	8	5.0	1.0	52	30	10.0	5	2.3	17	7.2	8	2.3	34	10.5
150.0	20	9.0	2.9				9	4.2			14	4.5		
0.0	18	22.0		80	45	15.4	19	8.8	49	24.7	30	8.7	61	20.0
0.0	25			74	50	17.4	17	7.8	43	22.8	27	7.4	68	22.4
0.0	3			74	50	17.0	22	9.1	53	28.8	18	4.9	71	28.5
0.0	0			70	42	15.0	23	10.3	50	27.7	22	6.7	64	22.9

TABLE XI

EFFECT OF SOURCE OF NITROGEN, PLANT GROWTH, AND COPPER SULPHATE TREATMENT ON pH OF SOIL CULTURES

COPPER ADDED TO AIR-DRY SOIL	CALCIUM NITRATE SERIES			AMMONIUM SULPHATE SERIES		
	pH AT SOIL MOISTURE CONTENT	SOIL MOISTURE	pH OF 1 : 5 SOIL-WATER SUSPENSION	pH AT SOIL MOISTURE CONTENT	SOIL MOISTURE	pH OF 1 : 5 SOIL-WATER SUSPENSION
p.p.m.	*pH*	%	*pH*	*pH*	%	*pH*
0.5	6.10	7.87	6.71	4.52	7.46	4.70
1.0	6.47	10.26	6.93	4.72	8.38	5.27
2.5	6.14	9.00	6.63	4.00	4.32	4.47
5.0	6.00	9.84	6.63	4.31	9.55	4.81
7.5	6.47	10.81	7.11	4.17	6.15	4.74
10.0	5.96	8.55	6.74	4.11	7.46	4.58
15.0	6.03	5.51	6.90	4.33	9.23	4.94
20.0	5.91	9.10	6.76	5.00	9.52	5.46
30.0	6.44	12.07	6.96	4.37	7.07	4.75
40.0	6.08	9.45	6.64	4.33	5.50	4.81
50.0	6.30	9.73	6.82	4.11	10.18	4.60
75.0	6.22	10.73	6.93	4.02	11.38	4.60
100.0	6.10	10.64	6.81	4.34	10.60	4.87
150.0	5.91	7.89	6.57	4.57	12.22	5.34
0.0	6.49	9.33	7.14	4.42	8.82	4.90
0.0	6.48	7.45	7.24	4.16	5.76	4.60
0.0	6.47	10.12	7.05	4.02	8.49	4.49
0.0	6.78	12.40	7.20	4.09	4.98	4.64

periments involving the use of copper sulphate in soil it is essential that the pH factor be controlled because the changes in pH may be of greater significance than the changes in the actual copper concentration in certain soils.

Many experiments have proved the more rapid growth of lemon cuttings in Hanford soil when ammonium sulphate was used rather than an equivalent amount of nitrogen in the form of calcium nitrate. On January 28, 1938, 3773 gm. (air-dry) Hanford soil was used in containers 6″ diam. × 6¾″ deep without drainage. A lemon cutting was planted in each culture and 0.365 gm. of nitrogen as calcium nitrate or ammonium sulphate was added. On December 7, 1938, the average twig length per culture in the ammonium-sulphate series was 48 inches and 40.3 inches per culture in the calcium-nitrate series. The leaves in the calcium-nitrate series were in poor health as compared with those in the ammonium-sulphate series.

Hydrogen ion determinations of composite dried cores of soil taken to the depth of the containers were: 6.90, 7.15, 7.18, and 7.36 for the calcium-nitrate series, and 4.94, 5.33, 5.35, and 5.71 for the ammonium-sulphate series. The cuttings in the best condition were those in the more acid series. Later, with lemon cultures grown out-of-doors, it will be shown that the acidity rather than the nature of the nitrogen is important for growth of lemon trees.

LEMON CULTURES CONTAINING TOXIC AMOUNTS OF BORON.—On February 4, 1938, 2-gallon crocks without drainage were used as containers for Hanford soil (9675 gm. air-dry) that was well mixed after receiving a solution containing 0.1014 gm. of boric acid. A Eureka lemon cutting was planted in each container. Subsequently, the cultures of one series each received (only initially) 15 ml. of dilute nitric acid solution (2 ml. concentrated nitric acid in 8 liters distilled water) as the source of nitrogen while those of the other series received an initial application of calcium nitrate solution containing 0.5 gm. of nitrogen.

It was noted that the cuttings in the nitric acid series grew rapidly but the leaves showed serious boron-excess symptoms with the burning and loss of many leaves. The cuttings in the calcium nitrate series showed only minor boron-excess symptoms and the cuttings appeared extremely healthy until the leaves began to turn yellowish green. The leaves in the nitric acid cultures also became yellowish green and showed very little if any boron injury in new leaves. The pH of (1:5 soil-water ratio) suspensions of the dried composite soil cores taken the full depth of each culture showed a pH range of 7.23–7.40 for the nitric-acid series and 6.93–7.20 for the calcium-nitrate series. Excessive initial acidity no doubt brought about boron injury because of high boron availability while calcium nitrate was useful in preventing boron injury. When the pH in the soil in each series of cultures became sufficiently high, boron-excess ceased to be a problem while the OH concentration became important. When boron is a factor in soil, a range of pH may be found in soil in which excellent growth may be obtained with the least boron injury.

In the present experiment, the addition of 40 p.p.m. copper as copper sulphate furnished sufficient acidity to cause a thorough greening-up of yellow leaves within less than one week with no evidence of boron-excess symptoms in the calcium-nitrate series. The calcium-nitrate cultures without the copper sulphate addition had a pH of 6.47 at a soil moisture content of 8.96 per cent. (dry-soil basis) with the spear-type glass electrode and 7.14 by the 1:5 soil-water ratio method. The calcium-nitrate cultures to which 40 p.p.m. copper as copper sulphate had been added, showed pH values about 6.00 at the soil moisture content of 6.34 per cent. and 6.94 by the 1:5 soil-water ratio method. Other pH determinations on calcium-nitrate cultures not copper-sulphate treated were 6.46, 6.49, and 6.45 respectively at the soil moisture content, and 7.34, 7.28, and 7.18 respectively at the 1:5 soil-water ratio. The copper sulphate solution was applied to the surface of the soil while the soil sample cores were taken the full depth of the containers. It is reasonable to conclude that the pH was lower near the surface of the soil than at the lower depths. The pH change brought about in soil when copper sulphate is added, may explain in part the response frequently found in some soils and not in others.

The effects of pH changes in soil on the growth of valencia orange cuttings is not as evident as with lemon cuttings but are equally definite.

VALENCIA ORANGE CUTTINGS.—On March 20, 1937, asphalt-coated tin containers 6″ diameter × 6¾″ high and without drainage, were given 4220 gm. (air-dry) of screened and well-mixed Hanford soil of the same lot as was previously used. Whenever nitrogen was applied, equivalent amounts (0.1825 gm.) were used from the following sources: calcium nitrate, ammonium sulphate, potassium nitrate, diammonium hydrogen phosphate, and ammonium citrate. Untreated control cultures also were used.

The application of large amounts of N/10 H_2SO_4 to certain of the soil cultures caused gum formation and subsequent death of the tops because of contact with the trunk bark, hence N/1000 H_2SO_4 was used in later treatments of these nitrogen cultures.

By June 8, 1937, the ammonium-nitrogen cultures had shown considerably more rapid growth than the nitrate-nitrogen cultures. On August 10, 1938, the untreated cultures were yellow and, while not making much if any new growth, were able to retain their leaves.

The calcium- or potassium-treated cultures made fair growth for a time and then were unable to retain many of the old leaves. In some cases the new leaves produced were severely mottled and in many cases the leaves were short and extremely narrow. The cultures treated with ammonium sulphate were the best and became so large that it was a problem to keep the leaves from wilting. The ammonium phosphate or citrate cultures were far superior to the nitrate cultures but were scarcely the equal of the ammonium sulphate cultures.

On August 28, 1938, core samples of soil were taken to the depth of the containers and the samples were composited for any one container, dried, screened, and mixed. The pH of the 5 or more cultures in each nitrogen series was determined by the glass electrode in (1:5 soil-water ratio) suspensions and the following ranges of values were found: Untreated, control cultures, pH 6.84–6.92; calcium nitrate, 7.30–7.73; potassium nitrate, 7.13–7.92; ammonium citrate, 5.78–6.10; diammonium hydrogen phosphate, 5.26–5.45; and ammonium sulphate, 4.49–5.00. The pH of the soil was found to be related to the growth produced.

VALENCIA ORANGE INJURY AND RECOVERY.—The photograph (fig. 10) taken February 14, 1939, illustrates the beneficial effects of increased acidity under the conditions of the experiment. The three cultures (fig. 10) were affected most by the continued use of calcium nitrate in the experiment just described and had pH values above 7.0. The cuttings were similar in appearance when selected for the recovery experiment. The two large cuttings to the left in figure 10 show the improvement in growth when N/500 sulphuric acid was used for irrigating the cultures as compared with distilled water alone.

FIG. 10. Valencia orange cuttings grown in Hanford soil that received calcium nitrate as the source of nitrogen. Right, no additional treatment, 2 cultures on left, dilute sulphuric acid $\left(\frac{N}{500}\right)$.

The leaf area of some of the valencia orange cultures was measured in April, 1939. A culture with potassium nitrate as the source of nitrogen had a leaf area of 54 1 square inches; another with calcium nitrate as the nitrogen source, had a leaf area of 117 7 square inches A culture that received calcium nitrate and later some sulphur had a leaf area of 199 5 square inches; another that received calcium nitrate and later some sulphur and dilute sulphuric acid had a leaf area of 286 9 square inches. These represent only a few of the many cultures grown that show good recovery upon lowering the pH of the soil.

The experiments cited thus far stress the importance of acidity in the growth of citrus. It has always been a question as to the source of the markedly beneficial action of ammonium sulphate in soil upon the growth of citrus. Experiments conducted out-of-doors with lemon cuttings in soil cultures, give additional evidence that the acidity produced by ammonium sulphate in soil is of value in addition to the nitrogen factor.

SOIL CULTURES OUT-OF-DOORS

LEMON CUTTINGS.—Out-of-doors, on a rain protected table in the lath enclosure, lemon cuttings were grown in asphalt-coated tin containers 6″ diameter × 6¾″ high, without drainage. A 4000 gm. sample (3939 gm. air-dry) of screened and well-mixed pasture soil, from the same lot previously used, was added to each container. A Lisbon lemon cutting was planted in the soil of each container on July 23, 1937. The cultures, except the controls, received 0.365 gm. of nitrogen as ammonium sulphate, potassium nitrate, or calcium nitrate. To 8 of the 16 cultures in each series at the start of the experiment, an amount of sulphuric acid was added in order to supply as much sulphate as occurs in the amount of ammonium sulphate used. No additional acid was used to equal the nitric acid that results from the transformation of the ammonium into nitric nitrogen.

On November 22, 1938, the lengths of the twigs were measured and it was found that the average total twig length of the 8 controls (checks) that received no nitrogen, was 29.2 inches while that for 8 other controls (checks) that received no nitrogen but received 251 ml. of 0.1046 N sulphuric acid at the start of the experiment was 33.9 inches. Similarly, the 8 cultures that received calcium nitrate but no acid had an average total twig length of 59.9 inches, while the average for the cultures that received acid was 64.5 inches. The 8 cultures that received potassium nitrate had an average total twig length of 59.9 inches while the average when acid was used, was 73 inches. This last value compares favorably with 71 inches average total twig length when ammonium sulphate was used without the addition of acid.

Other experiments are being carried out in this direction but they have not been operating sufficiently long to serve the present needs. The experiment just described, makes it appear that much of the value of ammonium sulphate over that of calcium or potassium nitrate in citrus growing may reside in the acid residues. Where alkaline residues can be reduced or readily leached away, calcium or potassium nitrates approach ammonium sulphate in value although leaching is necessarily wasteful of nitrogen.

Since lack of drainage in these containers prevents the removal of the bases, it is essential that acid be added to the cultures. Drainage was avoided in order to prevent losses of nitrogen which might affect growth. Likewise, all cultures were protected from rain in order not to saturate the soil and not to lose or gain nitrogen through splashing of rain against the soil.

From the experiments described, it is seen that the pH that favorably affects the growth of citrus is much lower than is ordinarily assumed. The determinations of pH under field moisture conditions, give a lower range of pH values than those made by the conventional (1:5 soil-water ratio)

method and give more nearly the actual pH of the soil in which growth takes place. The pH values of soils arrived at with the use of the spear-type glass electrode allows greater emphasis to be given to the results obtained with solution cultures. Determinations of the pH of soils at moisture contents above the soil moisture content in the field have caused a misunderstanding of the pH requirements of these trees and a lack of appreciation for results found (2) through the use of solution cultures.

Summary

The growth of citrus and other subtropical trees in artificial solution cultures was found to be affected by the pH of the medium in which the roots grow. The trees, while tolerating extremely low or high pH values in their nutrient medium, made greater and healthier growth in acid than in alkaline solutions. The degree of acidity at which growth is best, is more acid than is commonly believed.

The conception that these trees grow well at pH values as high as 8.5 or higher is based on faulty methods of preparing soil samples for pH determination. The pH of soils, as will be further elaborated upon in another report, depends upon the soil moisture content as well as on other factors and may vary considerably according to the soil moisture present at the time the determination is made.

When determinations of pH are properly made, it is found that the results obtained with artificial solution cultures as well as with soil cultures, agree very well with those obtained in the field.

The importance of pH for healthy growth has been emphasized. Several sources of nitrogen have been used together with various pH ranges in solution cultures. Equally important are the soil cultures in which the pH of the soil and its relation to the source of nitrogen has been determined.

The conclusion is reached that although trees in California are grown in the field under an irrigated agriculture, they are growing primarily in an acid rather than in an alkaline medium, and that the acidity of the medium is far greater than has previously been assumed.

Thanks are due Professor M. R. Huberty and Mr. W. E. Picker of the Division of Irrigation for suggestions regarding the plant-water relations and other phases of soils. Thanks are also due Mr. J. S. Millar, who assisted in the daily routine of the cultures.

Citrus Experiment Station
University of California

LITERATURE CITED

1. Bryan, O. C. Effect of soil reaction (pH) on citrus. Florida Grower 44(7): 6. 1935.

2. HAAS, A. R. C. pH for healthy growth in citrus. California Citrog. **23**: 158, 176, 178, 180, 181. 1938.
3. —————. Some nutritional aspects in mottle-leaf and other physiological diseases of citrus. Hilgardia **6**: 484–559. 1932.
4. —————. Root temperature effects on the growth of walnut and avocado seedlings. California Avocado Assoc. Year Book **1939**: 96–102.
5. HAAS, A. R. C. and HALMA, F. F. Sap concentration and inorganic constituents of mature citrus leaves. Hilgardia **5**: 407–424. 1931.
6. MCGEORGE, W. T. The determination of soil reaction under field conditions by means of the spear-type glass electrode. Jour. Amer. Soc. Agron. **29**: 841–844. 1937.
7. SHEAR, G. M. The effects of soil reaction on the growth and chemical composition of annual garden flowers. Virginia Agr. Exp. Sta. Tech. Bull. 63. 1938.

THE BALANCE SHEET OF METABOLITES FOR POTATO DISCS SHOWING THE EFFECT OF SALTS AND DISSOLVED OXYGEN ON METABOLISM AT 23° C.[1]

F. C. STEWARD, P. R. STOUT, AND C. PRESTON

(WITH TWO FIGURES)

Introduction

This paper presents a summary of the effects of various salt and oxygen treatments so chosen that they comprise a range of conditions of peculiar interest to the student of salt accumulation.

In any account of plant metabolism important processes may evade recognition unless a balance sheet of the principal metabolites can be prepared. Such balance sheets have rarely been attempted although the work of RAISTRICK *et al.* (**31**) on fungi is a notable exception. The work of RAISTRICK was concerned, however, with the use of organisms under standardized nutritional conditions to effect organic synthesis and transformations; it did not describe the effect on the metabolism of a given organism of a range of external and nutritional conditions. There is need of more attention to balance sheets of plant metabolism and especially so with reference to the vexed problems of respiration. Deviations from the familiar equations $C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674{,}000$ cal. and $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 25{,}000$ cal. often tacitly accepted as representative of plant respiration, are multiplying. The work of GANE and others (**11, 16, 17**) has demonstrated the production of volatile substances other than carbon dioxide and large discrepancies between respiration and the simple equations above may be encountered. For example, ARCHBOLD and BARTER (**1**) observed that the loss of sugar and acid in respiring apples exceeded by 17 to 30 per cent. the carbon dioxide evolved. On the other hand, DASTUR and DESAI (**10**) found that in the respiration of rice the carbon dioxide evolved exceeded the utilization of sugar and they believe that acids formed during the synthesis of protein form the source of the carbon involved. Again Lundegårdh (**23**) states that in excised wheat roots 30 to 60 per cent. more glucose disappears than is accounted for by respiration and he tacitly assumes that the discrepancy is a measure of the sugar used in growth. In experiments with excised roots HOAGLAND has observed cases in which a very rapid disappearance of sugar occurred and carbon dioxide production continued after the cells seemed to be free of sugar.[2]

[1] This is the second of a series of papers on The Biochemistry of Salt Absorption by Plants. The authors are indebted to PROF. HOAGLAND for proof-reading this paper.

[2] Results privately communicated.

From our present standpoint the outstanding fault in the above equations is lack of any implication that respiration concerns substances other than carbohydrates or that aerobic respiration and nitrogen metabolism are mutually dependent processes in plants.

The variation between replicate samples of standard potato discs is small (47) and the effect of metabolism on the constituents of the tissue can be obtained by difference between the composition of the initial and final, experimentally treated, batches of discs. It is possible, therefore, to draw up a balance sheet with reference to any metabolite, *e.g.*, carbohydrate, the fate of which is known with sufficient certainty so that its products can be determined. Balance sheets for dry-weight, calorific value, and total carbon may be similarly constructed and discrepancies in them would indicate an incomplete knowledge of the metabolic processes of the cells. Confidence that the effect of the various treatments on the metabolic processes of potato discs had been adequately comprehended was gained only when these various balance sheets appeared in mutual agreement and no longer showed discrepancies greater than the legitimate but small errors of the measurements. The balance sheets demand analytical data which are absolute and not merely of relative value. The methods already outlined (47) were carefully scrutinized and standardized. The concordant results from many different determinations obtained by the combined efforts of different individuals testify to the accuracy which has been attained.

Methods

Analytical procedures

The analytical procedures used in this paper and not hitherto referred to were the determination by wet combustion methods of the total carbon content of vacuum dry potato samples; the determination of the total uronic acid content of similar samples; and the measurement after their combustion to carbon dioxide of the volatile organic compounds produced by metabolizing potato discs. The wet combustions were carried out on re-dried samples (approximately 100 mg.) by a method used for determination of carbon in soils.[3] The method described by Raistrick (31) has also been used successfully, especially in connection with the removal of carbon from the discs by blotting. The uronic acid determinations were made by the method described by Dore in which the uronic acid is distilled with HCl and produces furfural plus carbon dioxide. The sample was heated under reflux condenser with 12 per cent. HCl for 4.5 hours. The carbon dioxide evolved in heating was absorbed in a potash bulb (after purification in the train of absorbents recommended by Dore) and deter-

[3] The addition of mercuric oxide was found to prevent the distillation of free bromine from the oxidation of tissue which contained bromide.

mined by weighing. The pectin value of the uronic acid found could be calculated knowing that each molecule of carbon dioxide collected corresponded to one molecule of uronic acid and assuming that this formed part of a tetragalacturonic-galactose-arabinose complex or its methyl esters (**7, 12, 13**). The volatile substances other than carbon dioxide which may be produced during the respiration of plants have recently received considerable attention. Such compounds were converted to carbon dioxide by passing the suspected gases through a heated combustion tube charged as in standard combustion practice with copper oxide (pure, from wire) and lead chromate.

The carbon equivalent of the volatile substances was obtained from the yield of carbon dioxide which was determined either volumetrically or by absorption in standard potash bulbs (pattern after CLASSEN) according to convenience. The variations in this basic procedure which were made to meet the needs of special experiments are described in the appropriate place in the text.

TREATMENTS OF THE POTATO DISCS

The general effect of salts and aeration on the metabolism of thin discs of potato tissue is shown by the effect of six different treatments as follows:

(1) Discs in distilled water at 23° C., aerated by a rapid stream of carbon-dioxide-free air.

(2) Discs in aerated dilute potassium bromide solution at 23° C. and of a strength (0.005 equiv. per liter) such that accumulation of the salt by the tissue would definitely occur.

(3) Discs in a relatively strong potassium bromide solution (0.05 equiv. per liter) aerated with carbon-dioxide-free air and at 23° C.

(4) Discs in dilute potassium bromide solution (0.005 equiv. per liter) at 23° C. but aerated with an oxygen-nitrogen mixture rich enough in oxygen (3.80 per cent.) to prevent break-down in the tissue, but too poor in oxygen to permit conspicuous salt accumulation.

(5) Discs in dilute potassium nitrate solution (0.005 equiv. per liter) aerated with carbon-dioxide-free air and at 23° C.

(6) Discs in dilute calcium bromide solution (0.005 equiv. per liter) and aerated with carbon-dioxide-free air and at 23° C.

In all the above cases the unspecified details conform to the requirements of the technique and apparatus previously described (**39**). The volume of the solution was two liters, the number of discs 60, and the duration of the experimental treatment 70 hours.

At the outset it will be clear that the comparison of the tissue from treatment no. 1 with the corresponding initial tissue, evaluates the effect on the various metabolites of those processes which are independent of

salt accumulation and which are determined mainly by the variables temperature, aeration, and time.

The tissues subjected to treatment no. 2 by comparison with that obtained from no. 1 tests the possibility that, even in the dilute solutions from which accumulation occurs, the ions of a readily accumulated salt cause specific effects on the metabolic processes.

Treatment no. 3 provides all the conditions necessary for optimum activity in the cells but a salt concentration which, though it would be conducive to the absorption of an increased *amount* of salt, would suppress the degree of *accumulation.* Specific effects of these readily absorbed ions on metabolism which are determined by the *quantity* of salt absorbed should be accentuated in treatment no. 3 compared to treatment no. 2.

Treatment no. 4 should be compared with treatment no. 2; the differences in the cultures are attributable solely to the oxygen concentration. At the lower oxygen tension it would be expected that any metabolic process with which the accumulation of salt is directly connected would be small.

Treatments nos. 5 and 6 evaluate the effect on metabolism of specific ions which have a special interest in the problem of salt uptake. Treatment no. 5 provides an anion (NO_3) which is absorbed in excess of even the readily absorbed cation (K); and treatment no. 6 provides by comparison with treatment no. 2 a case in which the uptake of even the readily accumulated anion (Br) is retarded by the cation calcium which is more slowly absorbed.

Results

Effect of salts and oxygen on the absorption of ions

Table I records the total absorption of anion and cation by 45 gm. (initial weight) of potato discs and shows that certain expectations were fulfilled.

The analyses were most conveniently made on the external solution. The bulk of this was rather large and therefore in calculating the total amounts absorbed error may be incurred. The absorption of the anions, however, was also obtained by analyses on the dried tissue. For the case of bromide (nos. 1, 2, 3, 4, 6) the agreement is so close that great confidence can be placed in the analyses on the external solution alone. The determination of the uptake of potassium from analyses on the tissue dried after blotting yielded figures of the same order as, but somewhat less than, those in table I which are preferred because the data obtained from the tissue are complicated by the effects of blotting the discs.

The potassium which may leave the tissue in the first few hours (38) after immersion was re-absorbed and at the end of the experiment the

TABLE I

EFFECT OF THE TREATMENT ON THE WATER AND IONIC RELATIONS OF TISSUE AND SOLUTION

TREATMENT		TOTAL MG. EQUIVALENTS ABSORBED BY 45 GM. OF ORIGINAL TISSUE			TOTAL MG. EQUIV. HCO_3 IN RESIDUAL EXTERNAL SOLUTION	FINAL pH EXTERNAL SOLUTION	PER CENT. GAIN OF FRESH WEIGHT
NUMBER	CONDITIONS	ANION DATA FROM EXTERNAL SOLUTION	ANION DATA FROM DRIED TISSUE	CATION DATA FROM EXTERNAL SOLUTION			
						pH	%
1	Dist. H_2O, air, 23° C.			0.02	0.20	5.6	7.27
2	0.005 equiv. KBr, air, 23° C.	1.3	1.12	1.40	0.36	5.9	8.09
3	0.05 equiv. KBr, air, 23° C.	2.4	2.34	5.00	0.28	5.9	4.09
4	0.005 equiv. KBr, air, 3.80 per cent. O_2, 23° C.	0.5	0.55	0.70	0.24	5.9	8.24
5	0.005 equiv. KNO_3, air, 23° C.	4.12*	1.66† 3.31‡	1.60	2.56	6.8	10.50
6	0.005 equiv. $CaBr_2$, air, 23° C.	0.96	1.04		1.04	6.8	6.46

* Loss of nitrate by external solution.
† Gain of organic nitrogen by tissue—not including NO_3 or NO_2.
‡ Gain of total nitrogen by tissue including NO_3 or NO_2.

tissue exposed to distilled water retained its initial salt content. At low oxygen concentration (solution in equilibrium with 3.80 per cent. O_2) the quantity of both bromide and potassium absorbed was much less than in similar solutions which were in equilibrium with air. A tenfold increment of salt concentration caused approximately a twofold increase in bromide absorption (**40**, p. 225). In the cultures of potassium bromide, bicarbonate did not occur in the final solution in amounts greatly in excess of the amount contained in distilled water in which tissue had respired for the same period. This, together with the fact that the final pH of the salt (nos. 2, 4) and distilled water (no. 1) cultures was approximately the same, is in accord with previous evidence that potassium and bromide are absorbed from *dilute* solution in equivalent amounts by potato discs. In the strong solution (no. 3) the cation (K) was absorbed in excess of the anion (Br) and from the work of STEWARD and HARRISON (**46**) two distinct mechanisms may be involved in the uptake of the cation. The excess absorption of cation over anion in the experiments referred to was due to an uptake of the former by a mechanism which is independent of vital processes (the so-called "induced absorption") and which, unlike the accumulation of potassium bromide ("primary absorption"), is not retarded in strong salt solutions. From a solution of calcium bromide (no. 6) a smaller bromide uptake occurred than from the corresponding potassium

salt (no. 2), the final solution became more alkaline and contained an amount of bicarbonate ion equivalent to the bromide removed. These facts indicate that much, if not all, of the bromide from calcium bromide was absorbed unaccompanied by cation and replaced in the external solution by bicarbonate ion (compare (**18**, p. 498)). Similarly, the nitrate ion was absorbed in excess of the cation (K) from a solution of potassium nitrate (no. 5) and the total uptake of anion was greater than that of bromide under otherwise similar conditions (no. 2).

The determination of the amount of nitrate absorbed by the cells is complicated by several factors. A *minimum* figure for nitrate absorption is given by the gain of total organic nitrogen by the tissue. This figure may be too low if nitrate remained unmetabolized in the cells as nitrate or nitrite. Both the gain of organic nitrogen and of total nitrogen including nitrate and nitrite were determined and, as shown by table I, even the latter figure (3.31 mg. equiv.) is less than the decrease of nitrate in the external solution (4.12 mg. equiv.). The latter figure may be in excess of the nitrate absorbed owing to reduction of nitrate by reducing substances potato tissue contains—substances[4] which were found in leached extracts of living tissue (**37**). In the light of the work of PEARSALL and BILLIMORIA (**28**) however, this discrepancy may be caused by decomposition of amino compounds and subsequent loss of nitrogen in molecular form in which case an equal amount ($4.12 - 3.31 = 0.81$ mg. equiv.) of amino nitrogen must also have disappeared from the tissue. In any event it is significant that the excess absorption of nitrate over potassium ($4.12 - 1.60 = 2.52$ mg. equiv.) is almost exactly balanced by the residual bicarbonate (2.56 mg. equiv.) in the external solution.

THE EFFECTS OF SALTS AND OXYGEN ON WATER ABSORPTION

There is an evident contrast between the effect on water absorption of treatments nos. 2 and 5 on the one hand and no. 6 on the other. In nos. 2 and 5, despite the presence of an external solute, the tissue gained *more* water than from water free from salt (no. 1) whereas treatment no. 6 produced an uptake of water significantly less than that of treatment no. 1. Reason may be sought in some specific effect of the ions and is not due to the osmotic value of the salts. Starch hydrolysis causes an increase of osmotic pressure in the sap but the final sugar concentrations (table III) which fall in the order treatment no. $5 < 2 < 1 < 6$, clearly cannot explain the water absorption figures which are in the order no. $5 > 2 > 1 > 6$. The same is true of the salt absorption data since by this criterion the calcium

[4] The reducing properties of these leached extracts are not attributable to their content of ascorbic acid, although they were first observed before the reactions of this substance became known.

culture again absorbs an unexpectedly small amount of water. The presence of potassium promotes water absorption in potato discs in aerated solutions whereas the presence of calcium depresses it and this is not due to *any known effect on the osmotic pressure of the cell sap.* The outstanding result of treatment no. 5 shows that when accompanied by nitrate the effects due to the potassium ion are accentuated. The same effect will later be evident on other aspects of the behavior of potato cells, *e.g.*, respiration and protein synthesis. These effects suggest that the water uptake of the cells is linked with the vital processes mentioned. Extreme modifications in the external and internal solute concentration do influence the water uptake as expected from simple osmotic theory. The comparison of treatments nos. 2 and 3 shows that after a tenfold increase in salt concentration the specific effect of the potassium salt is masked by the osmotic effect of the stronger salt solution. Similarly, at low oxygen concentration (treatment no. 4) though absorption is suppressed and the vital processes operate at a minimal rate the tissue still absorbed water; but this could be attributed to the fact that with reduced metabolism these cells retained a higher sugar concentration than other cultures (nos. 2 and 5) in potassium salts of the same concentration.

The water absorption of potato discs is not explained, therefore, wholly in terms of the osmotic value of the external solutions even the known factors (total salt and sugar concentration) which affect the osmotic pressure of the cell sap. Effects of salts on the permeability of the protoplast to water, of the kind which have been described by BAPTISTE (**2**) might affect the rate at which osmotic equilibrium is established but should not determine the total amount of water absorbed. Also, there is little experimental basis for the possible suggestion that the effects described could be due solely to effects on the properties of the cell wall.

The mechanism of water absorption involves a somewhat baffling array of variables many of which also affect metabolism. At the same equivalent salt *and oxygen* concentration, that treatment which caused the greatest protein synthesis (no. 5) produced the maximum water absorption *despite the fact* that it depleted the soluble reserves (sugar and amino-acids); whereas the treatment (no. 6) which, although it conserved the reserves of sugar yet depressed the synthesis of protein, also decreased the water absorption. Though the water absorption data here recorded are admittedly few they are supported by much other evidence and they refer to cells the metabolic history of which was extensively investigated. Therefore, despite the seeming over-emphasis, it is suggested that actively metabolizing cells which can grow, may absorb water in a manner which has but little relation to any conventional osmotic or suction pressure theory but may be more directly linked with metabolic processes (respiration and protein

synthesis); processes which are determined by oxygen and affected by the nature of the salts present in the external solution.[5] Relevant to this standpoint is the recent work of BENNET-CLARK *et al.* (5). These authors definitely contemplate the possibility that water absorption, like salt accumulation, is not merely an equilibrium process but may involve a mechanism of "internal secretion" which is peculiar to cells which are still able to grow. In this connection the brief statement of ERNST (14) that water may flow across a membrane against the hydrostatic gradient if there is maintained a temperature difference raises a possibility of the greatest interest.

Investigations of the water absorption of potato tissue have dealt with the effect of suction pressure (50, 51); the permeability, real or supposed, of the cells to water (2, 49); the use of water content as an index of toxicity [series of papers commencing with STILES (48)] or with the supposed connection between the colloidal swelling of ampholytes, particularly protein, and the water absorption of the cells (26, 27, 35, 36). Remarkably little attention has been paid to the variables which affect vital activity and metabolic processes, although an exaggerated emphasis has been placed upon certain small effects of external pH on water absorption, to the neglect of those variables (oxygen concentration, presence of neutral salts) which have little demonstrable effect on the swelling of colloids *in vitro* but do have a great effect upon water absorption and metabolic processes. That the colloidal properties of ampholytes may contribute to the water relations of cells can hardly be disputed but it seems equally evident that the rôle of the proteins and nitrogen metabolism in water uptake is a more intimate one in organized growing cells than the mere passive behavior which *in vitro* swelling experiments reveal.

The effects of nutrition on water content and nitrogen metabolism of growing plants emphasize that they are interrelated. This is shown by

[5] Since the above was written the paper of REINDERS (34) has appeared. REINDERS explores the possibility that water absorption, like salt absorption, is linked to vital activity—a standpoint for which there is much to be said. No conclusion is possible, however, from his data. It is true that REINDERS observes again, as one of us (F.C.S.) has often done in the past, that treatments which increase respiration in distilled water (and also salt uptake in salt solutions) also increase water absorption. The means by which REINDERS determined respiration (loss of dry weight) is however, excessively crude and, in the light of the various other processes in the tissue and involved in the technique of the experiments, is certainly misleading. As data on the actual concentrations of organic solutes (particularly sugar) in the experimental tissue is lacking (compare table III and fig. 2 of this paper) it cannot be concluded from REINDERS' paper that there is any discrepancy between the observed facts and the expectations of conventional osmotic theories of water absorption. It is of interest that dilute solutions of auxin influence the dry weight and the water uptake of potato discs; but this fact alone does not warrant the view that water uptake is anything more than a simple osmotic phenomenon.

the well-known relation between nitrogen supply and succulence, which was examined by PEARSALL and EWING (**27**). To cite but a few recent papers, those of GREGORY and SEN (**18**), confirming earlier results of RICHARDS (**33**), and PETRIE and WOOD (**29**) agree that nutritional treatments which during growth produce plants with a low net protein content also produce a low water content and, in a general way, the converse is true. PETRIE and WOOD discuss their data from the standpoint that water content determines the concentrations of nitrogenous constituents in the cells which in turn regulates the level of protein maintained; but in a final sentence they recognize the alternative view that "it is possible that the protein content is also a factor determining the water content to some extent." The results of tables I, II, and III suggest that aerobic respiration, protein synthesis, water absorption, and salt accumulation are all mutually dependent processes which occur in cells which are not subject to equilibrium conditions but the behavior of which, at constant temperature, is regulated by oxygen tension and the nature and concentration of the salt solution in which they are immersed.

EFFECT OF SALTS AND OXYGEN AT 23° C. ON RESPIRATION

Table II records the effect of the different treatments on respiration.

TABLE II

EFFECT OF SALTS AND OXYGEN UPON RESPIRATION

TREATMENT		RESPIRATION: RATES MG. CO_2 PER GM. HOUR		TOTAL* RESPIRATION CO_2
NUMBER	CONDITIONS	PERIOD 1	PERIOD 2	
		mg.	*mg.*	*mg.*
1	Dist. H_2O, air, 23° C.	0.204	0.220	683.0
2	0.005 equiv. KBr, air, 23° C.	0.180	0.237	706.0
3	0.05 equiv. KBr, air, 23° C.	0.222	0.289	867.0
4	0.005 equiv. KBr, 3.80 per cent. O_2, 23° C.	0.109	0.081	301.0
5	0.005 equiv. KNO_3, air, 23° C.	0.205	0.304	942.0
6	0.005 equiv. $CaBr_2$, air, 23° C.	0.178	0.188	640.0

* Corrected for bicarbonate content of the external solution.

The effect of oxygen, demonstrated elsewhere (**40** and references cited in **42** and **43**), is again shown by the comparison of treatments nos. 2 and 4. In the comparison of all other treatments (nos. 1, 2, 3, 5, 6) the considerable differences in respiration recorded (ranging from 640 to 942 mg.) were produced by the salt supplied. By oxygen concentration alone the respiration of potato tissue cannot be increased much above the value which it has

in distilled water in equilibrium with air although, owing to the external salt supply, increments of the order of 50 per cent. are here recorded at oxygen concentrations at which, on the former evidence, the respiration should be limited by factors other than oxygen. Clearly, therefore, the effect of salts on respiration is *through that factor, or group of factors, which in the earlier experiments was limiting at oxygen concentrations greater than those of solutions in equilibrium with air.*

The greatest response was obtained in dilute potassium nitrate and this suggests that the factor affected involves the nitrogen compounds. The outstanding effect of nitrate on respiration of potato discs, though somewhat unexpected in a tissue as rich in stored soluble nitrogen, is in agreement with many other recorded observations; an outstanding similar example being that recorded by HAMNER (19) in which large increments of respiration of high carbohydrate tomato plants occurred when nitrate was supplied. The comparison of treatments nos. 1, 2, and 3 indicates that as the total absorption of potassium bromide was increased there was a concomitant increase in carbon dioxide production. As in a previous paper (47) no such increase of respiration occurred in equivalent strengths (compare nos. 2 and 6) of calcium bromide; on the contrary, a significant decrease was observed. The cause of these salt effects on respiration must be sought in some factor which is affected reciprocally by the cations potassium and calcium and this is only apparent from the data on nitrogen metabolism (table IV and fig. 1).

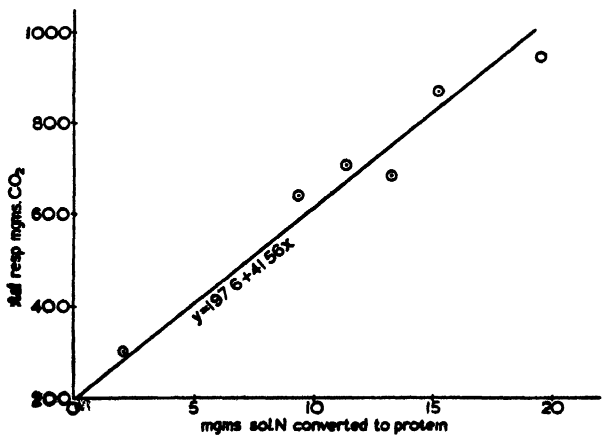


FIG. 1. Relationship between carbon dioxide respired and synthesis of protein in potato discs at 23° C.

It will be observed that the effects of salts on the respiration of potato tissue are distinct from, and opposed to, the view of LUNDEGÅRDH (24) who first ascribed all the effects of salts to the anions but who now admits that

there is a slight effect attributable to the cations although this appears only when there is intensive anion absorption (**23**). LUNDEGÅRDH further believes that the anions exert their effect through a special component of respiration which is a function of the anion actually absorbed. It will be noted that the cation effects are apparent in very dilute solutions and also that, in calcium bromide solutions (tables I, II) there was a considerable absorption of bromide which was accompanied by a *lower* respiration rate than that which obtained even in distilled water. The absorption data in table I and the total respiration figures in table II cannot be fitted into a scheme of the kind described by LUNDEGÅRDH or an equation of the form $R_t = R_g + KA$ where K is a constant specific for the anion and A the amount absorbed. This is apparent if the calculations are made from the data of tables I and II with reference to those treatments which involve only distilled water or bromides. Since the expression breaks down in its application to the bromide cultures *alone* there is no reason to discuss LUNDEGÅRDH's latest views (**23**) which concern the relationship between respiration as it occurs in salt solutions and in distilled water.

That salts and oxygen at 23° C. affect metabolic processes other than respiration is shown by their effect (tables III, IV, V) on the various metabolites of the discs. The analytical determinations, to which earlier reference has been made, were applied to the tissue and, where necessary, to the final external solutions. The data were calculated to the basis of 60 discs and 45 gm. of initial fresh weight (see also **47**).

EFFECTS OF SALTS AND OXYGEN AT 23° C. ON STARCH AND SUGAR CONTENT OF POTATO DISCS

The results of the carbohydrate determinations will be considered later in relation to other metabolites. First, certain general points need emphasis.

In all cases (see table III, treatments nos. 1 to 6) in which discs were exposed for 70 hours to the conditions used for the salt accumulation experiments they acquired sugar concentrations much in excess of their initial condition (table III, no. 7). The figures for starch determination show that very considerable quantities of starch disappeared—a fact which can be demonstrated microscopically (**53**).

Comparison of treatments nos. 2 and 4 shows that at the low oxygen concentration which retards the metabolism of the tissue, less hydrolysis of starch occurred. Potassium bromide cultures had no appreciable effect on the starch hydrolysis which was determined rather by time, oxygen, and temperature (compare nos. 1, 2, 3). Comparison of salt treatments nos. 2 and 6 shows that a calcium salt decreased starch hydrolysis.

The effects of the various treatments on the residual sugar concentration are clear. In cultures in equilibrium with air (nos. 1, 2, 3, 5, 6) the

TABLE III

EFFECT OF SALTS AND OXYGEN UPON STARCH AND SUGAR CONTENT DURING 70 HOURS AT 23° C.

Conditions		Starch content grams per 45 grams of original discs	Sugar concentration*	
Number	Treatment		Grams per 45 grams original discs	Grams in 2 liters of external solution per 45 grams of original discs
		gm.	*gm.*	*gm.*
1	Dist. H_2O, air	3.04	0.517	0.035
2	0.005 equiv. KBr, air	3.03	0.454	0.050
3	0.05 equiv. KBr, air	3.05	0.393	0.048
4	0.005 equiv. KBr, 3.80 per cent O_2	3.77	0.506	0.040
5	0.005 equiv. KNO_3, air	3.22	0.361	0.052
6	0.005 equiv. $CaBr_2$, air	3.51	0.568	0.056
7	Original washed discs	4.29	0.220	

* Calculated as glucose.

salt treatments which increase respiration (nos. 2, 3, 5) decrease the final sugar concentration. The increased sugar concentration which occurs at the cut potato surface has been regarded as the cause of the high respiration which this treatment produces (**22**). Clearly, under the above treatments, the *highest* respiration rates are associated with the *lowest* sugar concentrations (compare tables II and III). Thus sugar appears to be related to the respiration merely as the source from which carbon is drawn and not as the immediate respiratory substrate the *gross concentration* of which regulates the respiration rate.

It is possible, though perhaps improbable, that a detailed analysis of the individual sugars might reveal one more closely related to the respiration rate than the total sugar concentration. In the experiments of HOPKINS (*loc. cit.*) the proportion of reducing sugar to total sugar varied in different experiments from approximately 35 to 70 per cent. but within each experiment it remained constant and was subject only to casual variations. In other experiments[6] both the reducing sugar and disaccharide content of standard discs of potato after exposure to the same conditions of temperature and aeration as were used in the experiments of table III has been determined at various times during storage. The ratio hexose: sucrose varied from 0.98 to 1.4 with a mean at 1.14. Of the final sugar residue approximately half is hexose.

The relation of the respiration of whole tubers to their sugar content has been exhaustively investigated by BARKER (**3, 4**) who found that when high sugar content was produced by low temperatures this also caused the

[6] Unpublished data. Botany Dept., Birkbeck College.

accumulation of a respiratory inhibitor. The mutual inter-action of sugar concentration and inhibitor on the respiration rate is complex, but BARKER concludes that it is the sucrose concentration, not the total sugar concentration, which is most closely correlated with the respiration rate of whole tubers. In the treatments studied by DENNY *et al.* (**11**) the high respiration precedes the attainment of high sugar concentrations and these workers believe that organic acids (citric) are the immediate respiratory substrates. The point of view of this series of papers is, however, that the effects induced by salts on the respiration rate of potato discs are not attributable to their effect upon the concentration of sugars but to factors which become apparent only when the nitrogen metabolism is appreciated.

EFFECT OF SALTS AND OXYGEN ON NITROGEN METABOLISM AT 23° C.

The effect of the different treatments previously described on the alcohol soluble and alcohol insoluble nitrogen fractions is shown in table IV.

TABLE IV

EFFECT OF SALTS AND OXYGEN ON NITROGEN METABOLISM OF POTATO DISCS DURING 70 HOURS AT 23° C.

TREATMENT		SOLUBLE N AS PER CENT. OF TOTAL RECOVERED N	PROTEIN N AS PER CENT. OF TOTAL RECOVERED N
NUMBER	CONDITIONS		
		%	%
1	Dist. H_2O, air, 23° C.	50.0	50.0
2	0.005 equiv. KBr, air, 23° C.	51.9	48.1
3	0.05 equiv. KBr, air, 23° C.	47.6	52.4
4	0.005 equiv KBr, 3.80 per cent. O_2, 23° C.	61.4	38.6
5	0.005 equiv. KNO_3, air, 23° C.	67.2*	56.4*
6	0.005 equiv. $CaBr_2$, air, 23° C.	54.0	46.0
7	Original washed discs	63.5	36.5

* Expressed as percentage of initial total N.

Analysis of the external solution showed that the total amount of nitrogen in the two liters of solution was negligible. Hence the nitrogen effects can be dealt with by reference only to the tissue. The sum of the alcohol insoluble nitrogen fractions calculated to the basis of 45 gm. of initial tissue (60 discs) gives an estimate of their total nitrogen content. The combined variation in total nitrogen is due to inherent variability of the tissue and the errors of determining the nitrogen fractions. From seven determinations the mean total nitrogen content of 60 discs (45 gm. initial weight) was 98.6 mg. and apart from treatment no. 5, in which the tissue gained in total nitrogen by 23.6 per cent., the discrepancy between the total nitrogen recovered and the mean total nitrogen content never exceeded 2.5 per cent.

Treatment no. 1 shows that during 70 hours at 23° C. in aerated distilled water, the tissue synthesized protein (13.3 mg. of protein nitrogen per

45 gm. of initial discs) at the expense of the soluble nitrogen reserves. STUART and APPLEMAN (*loc. cit.*) have also recorded an increase, although very slight, of protein content in potato slices, which presumably had a very small specific surface, during wound periderm formation. As in our experience, the synthesis occurred at the expense of α-amino-nitrogen reserves. The synthesis of protein from stored soluble nitrogen compounds is clearly dependent upon oxygen (compare nos. 1 and 4); at the low partial pressure of 3.80 per cent. (no. 4) the loss of soluble and the gain of protein nitrogen was hardly significant.

The effects of salts, which are superimposed upon the effects of oxygen, are evident from the comparison of treatments nos. 1, 2, 3, 5, 6.

As expected, the nitrate treatment (no. 5) caused a gain of total nitrogen, of soluble nitrogen, and also a greater protein synthesis than in any other culture.

The calcium salt (no. 6) which depressed the respiration, carbohydrate metabolism, and water absorption, also retarded protein synthesis,[7] whereas the stronger potassium salt,[8] which increased respiration and the utilization of carbohydrate, also caused an increase in the synthesis of protein at the expense of the soluble nitrogen fraction.[9]

RELATION BETWEEN THE EFFECTS OF SALTS AND OXYGEN UPON RESPIRATION AND PROTEIN SYNTHESIS AT 23° C.

There is an evident parallelism between the effects on respiration and protein synthesis of the treatments investigated. Low respiration is associated with low protein synthesis (no. 4) and the highest respiration with the greatest protein synthesis (no. 5). Figure 1 shows graphically the approximately linear relationship between the total respiration–y (mg. of CO_2 respired, for data see table II), and the mg. of soluble nitrogen converted to protein–x, [gain of protein N (table IV) as percentage of total $N \times 98.6$ mg.]. The line drawn represents the closest fit and has the equation $y = 197.6 + 41.56x$. The conclusion follows that for this range of treatments, per mg. of nitrogen in the protein synthesized, the associated increase of respiration is equal to 41.56 mg. of carbon dioxide or, per equivalent of nitrogen converted to protein, an additional 13.2 mols of carbon dioxide

[7] The differences between treatments 1 and 6 are not large, but they are confirmed by a large amount of evidence which will be presented in a subsequent paper.

[8] The small differences between treatments 1 and 2 cannot be regarded as real since they have not been confirmed in the extensive experiments to be discussed later.

[9] These effects of cations should be clearly distinguished from the effects of acute mineral deficiency. In the cells concerned neither potassium nor calcium were at this low level. The fact that a calcium salt decreases protein synthesis in potato discs does not indicate that calcium is unnecessary for this process; when calcium is supplied at concentrations which limit growth, protein synthesis may be limited by the lack of calcium.

were formed over and above a basal carbon dioxide production which occurred independently of protein synthesis. From an arbitrarily selected range of conditions the arithmetical relationship between one equivalent of soluble nitrogen synthesized to protein and two (strictly 2.2) molecules of hexose aerobically oxidized to sugar should not be overemphasized. The important point is that the effects of salts and those of oxygen upon respiration are exerted through the same component of the total respiration and this component, moreover, is closely linked with protein synthesis.

The explanation of the connection between respiration and nitrogen metabolism is not immediately apparent from these results. A full discussion would entail a more extensive treatment of the literature of nitrogen metabolism, especially as it is affected by nutrition, carbohydrate supply, and oxygen, than can be included in this paper. Although it will be amplified in subsequent papers, the approach to the problem can be stated briefly for present purposes as follows.

The soluble nitrogen of potato discs consists of amino acids and amides (**47**). Usually amino acid predominates over amide and as shown previously it is from this fraction that the nitrogen for synthesis is derived. It is postulated that the amino acid liberates ammonia by oxidative deamination and that this process links protein synthesis, respiration, and the effect of salts and oxygen. As shown by RAPER and his colleagues (**32**), the effective oxidizing agent of the aerobic oxidase system of the potato is an ortho-quinone derived from catechol or a substance which contains this grouping and, from the work of HAPPOLD and RAPER (**20**), it is also known that this oxidizing agent deaminates amino acids (*e.g.*, glycine, alanine, phenylalanine). More recently DAMODARAN and NAIR (**9**) have described a specific aerobic dehydrogenase which converts glutamic acid to α-ketoglutaric acid; this enzyme has been demonstrated only in seeds. In a previous paper (**47**), it was shown that the treatments with inorganic salts and oxygen which affected respiration and protein synthesis similarly affected in a visible manner the activity of this oxidizing system within the living cells of potato discs. It follows from the large amount of carbon dioxide which accompanied the metabolism of each equivalent of nitrogen (fig. 1) that the carbon residue from deamination of the amino acids supplied a small part of the total carbon involved in respiration; and the balance, as also the carbon necessary for protein synthesis, must have been derived from sugar or organic acids or both. The large consumption of carbohydrate shown in table III offers ample evidence, if such were needed, of the metabolism of carbohydrate. Reference to the earlier paper (**47**), however, shows that organic acids are similarly consumed and in the balance sheet of carbohydrates (**42**), the carbohydrate used was accounted for without assuming that it was converted to protein.

Hence sugar and amino acids bear to respiration and protein synthesis a similar relationship. They represent the sources from which the nitrogen and carbon are drawn, their *individual concentrations do not regulate respiration* but their utilization is dependent upon the activity of the same oxidizing system which is in turn regulated by the presence of salts and oxygen. This somewhat unconventional point of view finds its closest parallel in the interesting account given by GREGORY and SEN of the respiration of barley leaves subjected to different levels of mineral nutrition (**18**). A close comparison of the case of barley leaves and potato discs and a more detailed discussion of the relation between nitrogen metabolism and respiration will be deferred until more effects of salts on these processes have been presented in a later paper and until the effects of the ions on the components of the soluble nitrogen have been investigated. The oft cited work of SPOEHR and MCGEE focussed attention on the effect of amino acids on respiration. Since then the relation between nitrogen metabolism and other physiological functions, especially photosynthesis and respiration, and the way this is modified by other inorganic nutrients (*e.g.*, K) has become a major problem [see reviews of mineral nutrition of plants in ANNUAL REVIEWS OF BIOCHEMISTRY and a recent review by NIGHTINGALE (**35**)]. Our purpose here is to establish the connection between nitrogen metabolism and respiration for potato discs, to emphasize that it is the processes of synthesis from amino acids which are concerned in this relationship, and to postpone the fuller discussion of this problem and the relevant literature which is necessary.

THE COMPONENT OF RESPIRATION NOT LINKED TO PROTEIN SYNTHESIS

There is, however, a large part of the total respiration of potato discs at 23° C. which does not appear to be linked with protein metabolism and this can be estimated from the equation of figure 1 as the value of the total respiration ($y = 197.6$ mg. CO_2) at zero protein synthesis ($x = 0$). Expressed as an average rate this yields the figure 0.062 mg. CO_2 per gm. initial fresh weight per hour which can represent only the value of that component of the total carbon dioxide production which is not affected by the salt and oxygen treatment, is not related to the metabolism of the nitrogen compounds, and which earlier papers have shown is not concerned in salt accumulation (**44**). An independent estimate of the value of this component of respiration may be made from the effect of oxygen tension upon the respiration of similar potato discs at 23° C. which respired for the same total time period (70 hours) in distilled water or salt solutions so dilute that the specific effect of the salt can be neglected (**40**). If the respiration of potato discs may be partitioned into two components, the one linked to protein synthesis and the other independent of it, then the former should

vanish at zero oxygen tension and the probable value of the latter may thus be derived from the value which the respiration approaches at zero oxygen pressure (see (40), fig. 2). Hence the component independent of nitrogen

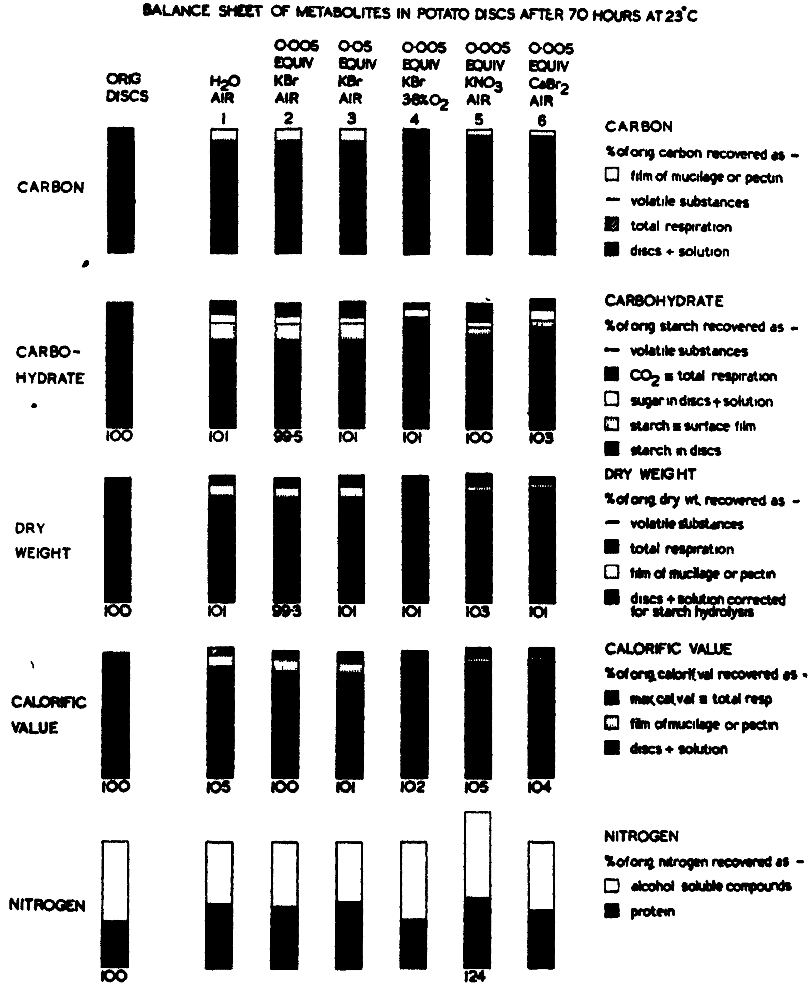


FIG. 2. Balance sheet of metabolites in potato discs at 23° C.

metabolism is estimated at 30 per cent. of the respiration of discs in dilute solutions in equilibrium with air at 23° C. and, for the case in question, would correspond to a rate of 0.072 mg. CO_2 per gm. per hour, a figure which agrees closely with that of 0.062 mg. per gm. per hour obtained above. This agreement can hardly be fortuitous and must surely mean that approximately one-third of the respiration of standard potato discs at 23° C. in distilled water or dilute salt solution is attributable to a respiratory

component which is not directly affected by variations in *oxygen concentration,* is not related to the nitrogen metabolism, or affected by the presence of salts and is not a factor in salt accumulation. The remaining two-thirds of the total respiration of the discs at 23° C. clearly represents that component which is influenced by salts, by oxygen concentration, and which is intimately concerned both with the mechanism of protein synthesis and salt accumulation of potato discs.

It is of the greatest interest that since the above was first written BOSWELL *and* WHITING (8) *have investigated the relationship of the respiration of potato discs to the polyphenol oxidase which they contain. Their conclusion is that the respiration consists of two components, both of which have an R.Q. = 1.0. One of these components contributes 66 per cent. of the total respiration and is brought about by the oxidation of a phenolic body to its oxidized form by oxidase and subsequent reduction, by a hydrogen donator, with the production of compounds which split off carbon dioxide. The identity of this respiratory component of* BOSWELL *and* WHITING *with that described above which is linked with protein synthesis seems hardly to be questioned. The oxidase and phenolic substances play an essential role in both and contribute the same proportion (⅔) of the total respiration.*

THE BALANCE SHEET OF METABOLISM

Thus far the way in which the metabolism of potato discs is affected by salts and aeration has been described. The recovery of the total metabolites may be tested in several ways. Each culture contained 60 discs from which duplicate and random samples of 30 discs were obtained and used for the determinations of dry-weight, sugar, starch content, calorific value, and carbon content. These determinations were also repeated on "initial discs" and all analytical data calculated on the basis of 45 gm. of initial fresh weight of washed discs. Since the total respiration was known and the small amount of organic matter present in the external solution was measured, a balance sheet may be prepared with respect to dry weight, carbohydrate calculated in terms of starch, calorific value, and carbon. This method enables the absolute recovery of the various metabolites to be measured.

Since the complete analytical technique was applied to two batches (A and B) of 30 discs random sampled from the 60 discs of each culture, all the determinations were carried out at least in duplicate. Where great certainty was desired replicate determinations were made on each of the samples A and B. From the values recorded for samples A and B the mean value for the tissue in each culture was obtained and this, when converted to the initial fresh weight basis, was the figure used in the balance sheet calculations. The duplicate tissue samples permit the effect of the

very small variability of potato tissue to be assessed. The full data will not be given here but it may be stated that the errors incurred by the variability of the material are small compared to the differences to which significance is attached (see also **47**).

To the observed final dry weight of the tissue was added a small correction (of the order of 0.040 gm.) which represents the dry weight of the organic matter in two liters of external solution and, unless the metabolite in question was directly determined (*e.g.*, sugar, total dry matter), it was assumed that this had the same percentage composition (heat and carbon content) as the dry material of the tissue sample. The estimate of organic dry matter in the external solution was obtained by the loss of dry weight[10] when an aliquot part was ignited and, especially in cultures nos. 3 and 6, it was necessary to correct this figure for the loss of weight sustained by a parallel sample of the same salt content, but free of organic matter, which received identical treatment. Under the conditions of drying, calcium bromide is hydrated and after ignition is anhydrous, whereas potassium bromide loses appreciable weight on ignition owing to the volatility of this salt. The corrections applied compensate for these sources of error.

Certain assumptions must be made in the balance sheet calculations. The expected effect of respiration on the total dry weight, carbohydrate, and heat content of the tissue can be estimated only in accordance with the familiar equation:

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O + 674{,}000 \text{ cal.}$$

despite the emphasis in an earlier section on the linkage between the respiratory processes and nitrogen metabolism which this equation does not reveal. The balance sheet method therefore tests whether the relationships between respiration and nitrogen metabolism involve drastic departures from this fundamental equation or only concern the intermediate steps by which the process is achieved. It has been assumed that the direct effect on dry weight, heat content, and carbon content of protein synthesis from soluble nitrogen compounds is negligible. Also, the effect of the reactions which involve organic acids on these properties was not evaluated. The significant effect on the dry weight caused by the hydrolysis of starch to sugar can be calculated from the observed gain in sugar content. The actual dry weight of the salt absorbed is small and has been neglected for present purposes. Within these limitations the results of table V lead to the following conclusions.

A complete account (accurate to 1 per cent.) of the fate of the dry weight, starch, heat, and carbon content of potato discs during their respi-

[10] For an estimate of the carbon present in the external solution reference may be made to later experiments (table VIII). For the proportions of tissue to solution in the above experiment this figure corresponds to 0.010 gm. of carbon in the external solution.

TABLE V

RECOVERY OF DRY WEIGHT, CARBOHYDRATE, AND CALORIFIC VALUE IN POTATO DISCS AFTER 70 HOURS OF METABOLISM AT 23° C. ALL QUANTITIES EXPRESSED PER 45 GRAMS (60 DISCS) OF INITIAL TISSUE

Treatment: Number	Treatment: Conditions	Dry weight of discs*	Dry weight loss by respiration	Gain of dry weight by starch hydrolysis†	Starch content	Starch used in respiration	Starch equivalent to gain of sugar‡	Heat content‡	Heat equivalent of respiration	Carbon content‡	Carbon equivalent to CO_2 respired	Percentage recovery of initial: Dry weight§	Starch	Heat	Carbon
		gm.	gm.	gm.	gm.	gm.	gm.	kgm. cal.	kgm. cal.	gm.	gm.	%	%	%	%
1	Dist. H_2O, air	5.52	0.413	0.033	3.045	0.417	0.300	22.88	1.73	2.080†	0.185	92.3	87.9	95.6	89.6
2	0.005 equiv. KBr, air	5.40	0.421	0.028	3.026	0.421	0.257	21.95	1.75	2.073	0.193	90.6	86.5	91.9	89.5
3	0.05 equiv. KBr, air	5.42	0.512	0.022	3.050	0.513	0.200	21.53	2.13	2.033	0.228	92.4	87.8	91.8	89.5
4	0.005 equiv. KBr, 3.80 per cent. O_2	6.26	0.190	0.033	3.775	0.186	0.294	25.26	0.77	2.415	0.083	100	99.5	101	99.0
5	0.005 equiv. KNO_3, air	5.74	0.575	0.019	3.218	0.575	0.174	23.81	2.02	2.124	0.256	98.1	92.7	100	94.2
6	0.005 equiv. $CaBr_2$, air	5.96	0.384	0.040	3.506	0.384	0.364	24.34	1.60	2.235	0.170	99.0	99.4	100.5	95.0
7	Initial tissue	6.40	......	......	4.286	.	.	25.80	......	2.528	......	......	......	......	......

* Calculated from sample "B" only.

† Calculated from gain of total sugar in final tissue over original discs. To correct for probable proportion of disaccharide to total sugar (50 per cent.) reduce these figures by 25 per cent.

‡ Values corrected for the organic matter contained in the external solution. Sugar content of external solution by direct determination (table III). Dry weight of organic matter in external solution by loss of weight on ignition minus the change in weight of a parallel sample which contained the same amount of the principal salt.

§ Dry weight changes which are the direct result of salt absorption are here neglected. These are small and lacking complete data, for all the inorganic ions, are somewhat difficult to evaluate. The maximum correction could be calculated assuming equivalent uptake of anion and cation from the figures of table I but this is obviously too large because some salts of calcium and magnesium do leave the tissue in the early stages of these experiments (38) and are not reabsorbed completely. Protein synthesis cannot affect the dry weight of balance appreciably. The total dry weight of protein synthesis used in no. 1 amounted to little more than 5 per cent. of the total initial dry weight and clearly the change in dry weight during synthesis would be only a small fraction of this.

ration and metabolism at a low oxygen concentration (no. 4) and in contact with potassium bromide was obtained by the methods described. Similarly when respiration occurs in solutions of calcium bromide in equilibrium with air, the balance sheet shows a satisfactory account of the principal metabolites. This is not so, however, in the case of treatments 1, 2, and 3 for there are evident discrepancies in the balance sheet with respect to dry weight, starch, heat, and carbon recovery which far exceed the legitimate errors. These discrepancies cannot be attributed to any of the metabolic processes as yet described and they clearly indicate that a considerable portion of the stored starch of discs which are exposed to aerated water or potassium bromide is converted to a form such that under the conditions of the storage tissue experiments the *bulk* of the heat, dry weight, and carbon it contained is *lost entirely by the tissue system* (see table VI).

TABLE VI

DISCREPANCIES IN THE BALANCE SHEET FOR STARCH, DRY WEIGHT, AND CARBON

Number	Initial dry weight not recovered	Initial starch not recovered	Initial carbon not recovered	Starch $(C_6H_{10}O_5)_n$ equivalent to carbon lost
	gm.	*gm.*	*gm.*	*gm.*
1	0.493	0.518	0.263	0.593
2	0.601	0.578	0.266	0.600
3	0.486	0.523	0.266	0.600

Confidence in this unexpected result is not merely based on the agreement between the independent results for dry weight, starch, heat, and carbon content in tables V and VI but it has been confirmed in several similar experiments. From the data obtained on the parallel samples, A and B, of each culture and by the method of the "t" test (15) the probability that the observed difference between the mean values for initial and final discs could have been due to chance may be estimated. The data for calorific value yielded the figures in table VII and these show that the discrepancies in the "balance sheet" with respect to calorific value are real, a conclusion

TABLE VII

CALORIFIC VALUE. SIGNIFICANCE OF THE DISCREPANCY IN THE "BALANCE SHEET"

Treatment		T	P
Number	Conditions		
1	Dist. H_2O, air	18.4	< 0.01
2	0.005 equiv. KBr, air	22.0	< 0.01
3	0.05 equiv. KBr, air	11.0	< 0.01

which also applies to the similar discrepancies in the recovery of starch, dry weight, and carbon.

To sum up, therefore, there still remains an outstanding aspect of the metabolism of potato discs which occurs under the experimental conditions conducive to high respiration and salt absorption and which has not yet been evaluated. This metabolic process is one which causes, under the conditions of these experiments, almost the entire disappearance of the heat, dry weight, and carbon content of the carbohydrate involved. Apparently the reaction concerned demands a high oxygen tension, is suppressed in calcium salts, and in some degree by the nitrate ion (table V).

Fate of the unrecovered dry matter

It may be stated now that a detailed investigation of the volatile substances produced during the metabolism of potato discs revealed amounts of only a much lower order of magnitude than the outstanding losses of carbon evident in table VI.

Investigation showed that the bulk of the organic substance which was missing (table V) was removed from the surface of the discs on the filter paper used for the removal of surface water. Incidental to the technique of the salt accumulation experiments, discs in distilled water or potassium salts are slightly mucilaginous at the surface *before* they are blotted but this disappears when they are surface dried. This effect is somewhat more pronounced in the case of discs exposed to potassium salts than in those from distilled water cultures but is not observed in the *initial discs* which were cut and washed in running tap water.

Experiment showed the amount of carbon which may be removed from the surface of discs by drying paper and also that, if this loss of carbon is appreciated, a satisfactory account of the total carbon content of discs respiring in distilled water may be rendered.

Two parallel cultures (nos. 1 and 2, table VIII) of 40 discs were exposed to aerated distilled water at 23° C. for 90 hours. Each culture was then random sampled into two batches of 20 discs one of which was surface dried; the other was merely rinsed. Subsequently all the samples were vacuum dried and their dry weight and total carbon content (wet combustion) determined. On an aliquot part of the external solution the amount of carbon it contained was determined and the initial carbon content (812 mg.) was obtained on four replicate batches of twenty discs random sampled from the same stock. The total respiration during the 90 hours of the experiment was measured in the usual way.

Table VIII shows that the discs which were surface dried had lower dry weight and carbon content than the corresponding sample which was not blotted and that these differences are far greater than the differences

TABLE VIII

EFFECT OF BLOTTING THE DISCS ON THE RECOVERY OF TOTAL CARBON

Treatment			Dry weight per 20 discs	Carbon per 20 discs	Carbon respired per 20 discs	Carbon in external solution	Total carbon recovered per 20 discs	Percentage recovery of initial carbon	
Number	Conditions	Tissue							
			gm.	*mg.*	*mg.*	*mg.*	*mg.*	%	
1	Dist. H_2O, 23° C.	Blotted	1.752	674	82.5	3.3	760	93.6	92.9
2	" " "	"	1.687	662	83.6	3.3	749	92.2	
1	" " "	Unblotted	1.971	719	82.5	3.3	805	99.2	98.6
2	" " "	"	2.078	708	83.6	3.3	795	98.0	

between duplicate cultures. Table VIII also shows that whereas in the blotted discs there was a 7 per cent. discrepancy between the initial and final recovered carbon (which has a significance of P = 0.01) in the carbon "balance sheet" this was reduced to 1.4 per cent. (which by the "t" test (18) is not significant since P = 0.4) when the surface drying on filter paper was omitted.

NATURE OF THE SUBSTANCES REMOVED BY BLOTTING THE DISCS

From the data of tables V, VI, and VIII it is clear that the substance which was removed on the filter paper was produced at the expense of the starch fraction and contained the entire carbon content and at least 75 per cent. of the calorific value of the starch from which it was formed. Furthermore, it must have been a substance capable of forming a superficial, mucilaginous film on the external surface of the discs. The complete recovery of carbon compounds obtained in table V in the low oxygen culture (no. 4), the better recovery (no. 6) obtained if the tissue was exposed to solutions containing calcium rather than potassium even though these were in equilibrium with air, (as well as the complete recovery of total nitrogen which has repeatedly been obtained, even where the losses of dry weight occurred) all show that the substances in question *could not have been removed from within the cells by the slight pressure necessary to remove superficial water.*

The formation of a surface deposit on potato slices exposed to moist air is a familiar fact. The cut surface becomes blocked with a mucilaginous substance and the superficial cells quickly suberise (30). Although it was possible that the fatty substances which are concerned in suberization contributed to the surface film of carbon compounds which adhere to the filter paper, this was dismissed when a direct comparison of the fat content of blotted and unblotted discs was made. The probability that the substance removed was a pectin or mucilage directed attention to the uronic acid content of potato discs and the effect upon it of surface drying by filter paper.

As in the experiment of table VIII, discs were exposed to 2 liters of aerated distilled water at 23° C. for 67 hours in two cultures (nos. 1 and 2) each of 40 discs. The final tissue from each culture was sub-divided into two random samples (20 discs) one of which was blotted dry, the other merely rinsed, before being vacuum dried. On the dried ground sample the uronic acid content was determined by the method of Dore and the corresponding pectin content calculated. Table IX shows that a significant decrease in the uronic acid content of the blotted discs occurred and also that, on the basis of the somewhat arbitrary composition assigned to the pectin "molecule," such a substance could account for at least 57 per cent.

TABLE IX

REMOVAL OF DRY WEIGHT AND URONIC ACID DERIVATIVES FROM POTATO DISCS AFTER EXPOSURE TO AERATED SOLUTION AT 23° C.

Treatment			Pectin* per 20 discs	Pectin* removed by blotting	Dry weight removed by blotting	Carbon removed by blotting 20 discs	
Number	Experimental conditions	Tissue				Observed by total carbon determination	Pectin removed
			mg.	*mg.*	*mg.*	*mg.*	*mg.*
1	Dist. H_2O, air	Blotted	48.9				
2	" " "	"	49.3	174	305	50	39
1	" " "	Unblotted	226.0				
2	" " "	"	221.0				

* Calculated from decrease in uronic acid as a tetramethylated-tetragalacturonic acid-galactose-arabinose compound.

of the actual dry weight which was removed by blotting the discs and as much as 80 per cent. of the carbon removed. If, as is often the case, the particular pectin involved had a lower uronic acid content, or was associated with appreciable quantities of inorganic ions (*e.g.*, calcium and magnesium), the weight of dry matter per unit of uronic acid might be considerably greater. In fact, in earlier experiments it was noted that aerated potato discs in distilled water or potassium bromide solutions did lose surprisingly large amounts of magnesium which it was presumed accompanied organic anions into the external solution (38). In the light of this later evidence it is probable that much of the magnesium lost by the tissue accumulated with pectin or mucilage as a film at the surface of the discs and it adhered to filter paper when the discs were surface dried; in fact, the amounts of magnesium lost in the earlier experiments were of the right order assuming that each equivalent of uronic acid removed by the blotting technique (table IX) was associated with an equivalent of magnesium. The accumulated evidence is strongly in favor of the view that much of the magnesium lost by the tissue in these earlier experiments disappeared combined with uronic acids in a pectin-like complex.

It is clear, therefore, that during the metabolism of potato discs at 23° C. in aerated solutions some substance, which in the initial tissue was determined as starch, was converted to pectin-like substances which *accumulated as a film at the surface of the discs.* In running tap water or low oxygen cultures at 23° C. this did not occur. In potassium bromide solutions, or distilled water, the film formed was relatively fluid and adhered to filter paper, whereas in contact with calcium salts, if the film

formed at all, it remained precipitated on the discs in a form not removed by contact with paper. In the conversion of carbohydrate to uronic acid, oxidation is involved and it is noteworthy that treatment no. 4 of table V shows that at low oxygen tension (3.80 per cent O_2) even in the presence of potassium bromide solution the losses now attributed to the pectin-like substances did not occur. If the salivary extract used for starch hydrolysis also hydrolyzed the pectin-like compound[11] the data would not demand an actual synthesis of the complex compounds of uronic acid from starch but merely that at 23° C., in the *presence of oxygen* and the relative absence of calcium, pectin compounds already present became mobile and accumulated at the surface of the discs as a film. There is no obvious explanation of the apparent effect of nitrate which tended to depress the loss of pectins except that this ion stimulated carbohydrate metabolism and in consequence reduced the residual sugar concentration available for other reactions.

With this unexpected aspect of the metabolism of immersed potato discs the general picture of the metabolic processes which involve carbohydrate is almost complete. The only fractions not yet measured quantitatively are the volatile ones and these, which are produced in amounts too small to affect the "balance sheet" greatly, will be discussed in the next section.

Volatile carbon compounds produced by the potato discs at 23° C.

An intensive investigation of the volatile substances produced by potato discs under the conditions conducive to salt accumulation was undertaken by C. Preston, but this can be only briefly summarized.

During the salt accumulation experiments volatile substances could be lost by the system at three stages in the technique, namely: (a), with the respired carbon dioxide in the air stream used for aeration; (b), with the water evolved during the vacuum drying of the tissue; and (c), during the evaporation of the external solution.

The carbon compounds evolved along with the carbon dioxide were converted to carbon dioxide by passage through a three-foot combustion furnace packed as in standard combustion practice. During 70 hours the respiration rate of the tissue in dilute salt solution remained very constant (see (47) for form of time curve). Comparisons were made between the total carbon dioxide (CO_2 of respiration + CO_2 from volatiles) from one culture and the respired carbon dioxide alone from another. Similarly on the same culture the respiration rate alone, during periods when the volatiles were not burned, was compared with the total yield of carbon dioxide during periods when the volatiles were passed through the hot furnace. Elaborate blank experiments evaluated the traces of carbon dioxide obtained from volatile compounds which were not evolved by the tissue.

[11] See footnote 12.

The mean rate of total carbon dioxide production (0.205 mg. per gm. per hour) was greater when the volatiles were burned than in a parallel culture in which this was not done (0.189 mg. per gm. per hour). It was also greater than the mean rate (0.195 mg. per gm. per hour) of carbon dioxide produced by the same culture in periods when the furnace was cold. Differences between the respiration rate alone of parallel cultures or differences between the mean rates of the control culture from two series of alternating periods were not significant when tested by the "t" test. The extra carbon dioxide due to the combustion of the volatile substances (0.010 mg. per gm. per hour) on the contrary represents a real difference ($S = 4.9$, $n = 4$, $t = 2.5$: P somewhat > 0.05) especially since the only point at issue is whether the combustion technique caused a significant *increase* of carbon dioxide and, therefore, the value of P could be legitimately halved ($P = 0.025$). Therefore, the loss of carbon by volatile substances swept away with the air stream is equivalent to only 0.010 mg. CO_2 per gm. per hour, or approximately 5 per cent. of the respiration rate.

Investigation with carefully controlled blank experiments showed that no significant amount of combustible carbon compounds was evolved during evaporation of the external solution in which potato discs had been placed at 23° C. During 120 hours at 23° C. in aerated solution 115 gm. of standard discs accumulated volatile substance equal to 18.2 mg. of carbon which was evolved during vacuum drying. Hence the volatile substance accumulated in the tissue and the solution during 120 hours of metabolism at 23° C. did not exceed the equivalent of 0.0048 mg. of CO_2 per gm. per hour.

The maximum amount, therefore, of volatile compounds formed by potato discs at 23° C. in aerated salt solutions can be assessed as follows:

CO_2 equivalent to combustible compounds in air stream	= 0.010	mg. per gm. per hour
" " " residual volatile compounds in tissue and external solution	= 0.0048	" "
" " " total volatile compounds produced	= 0.015	" "

The carbon dioxide equivalent of the total production of volatile compounds by standard potato discs at 23° C. is, therefore, approximately equal to 7.5 per cent. of the carbon dioxide evolved. An allowance of this amount for "volatiles" would increase the percentage of the total carbon accounted for in the case of unblotted tissue (table VIII) from 98.6 to 99.4 per cent.

Discussion

The corrected balance sheet of metabolites

A more complete balance sheet of the metabolites of potato discs showing the effect of the various treatments previously described may now be

prepared. The recovery of the initial carbon obtained in the discs plus solution and as respired carbon dioxide has been shown in table V. It has been proved that volatile carbon compounds accounted for only a very small amount of the unrecovered carbon (7.5 per cent. of the carbon evolved as carbon dioxide) and also that the carbon still unaccounted for was removed from the surface of the discs as a film of mucilage or pectin which adhered to the drying paper. The amount of carbon removed on the paper may be calculated from the difference between the initial carbon content of the discs and the final carbon recovered in the discs and solution plus the carbon value of the respiration and the volatile compounds evolved (equivalent to 7.5 per cent. of the carbon dioxide of respiration). Knowing the carbon removed by the blotting technique, its equivalent of starch, dry matter, or calories may be calculated since the parent substance was present in the initial discs in a form which was estimated as starch and, when the film was removed, the discs retained none of the carbon, dry weight, or heat of this substance (table VI).[12] The results of these calculations are set out in table X; in table XI the percentage recovery of the initial metabolites is given.

Reference to table XI and to the detailed figure 2 shows that the metabolic processes of potato discs have now been adequately comprehended and the fate of the principal metabolites estimated quantitatively. The account now rendered of the original carbohydrate and dry matter is accurate to 1 per cent. (except in two cases where the total discrepancy is 3 per cent.) despite the varied treatments to which the tissue was subjected and the diverse metabolic processes involved.

Source of carbon for protein synthesis

It is important to recognize that the balance sheet of carbohydrates is complete without any actual allowance for carbon which is drawn from sugar and built into the protein molecule. In other words, if the equivalent of the carbon content of the stored soluble nitrogen compounds is inadequate it must be supplemented from sources other than sugar (*e.g.*, organic acids which also disappear when protein is synthesized (17)).

[12] If the salivary extract used for starch hydrolysis also hydrolyzed the uronic-acid complex designated pectin, then from the standpoint of the balance sheets it would be immaterial whether the uronic-acid complex removed by blotting was synthesized from starch during the treatments or merely rendered mobile in the presence of oxygen, potassium bromide, and a temperature of 23° C., so that it accumulated as an adhesive film at the surface. Lacking the definite proof of this point for potato pectin it will be convenient to write of the pectin-like substance as though it were in fact synthesized during the treatment from the starch reserve. This can be justified from data on commercial apple pectin. A stronger salivary extract than that used for the starch hydrolyses (10 ml. saliva + 10 ml. water) yielded no more sugar after 24 hours incubation at 25° C. with 0.10 gm. of pectin than the original sample contained (0.010 gm. reducing sugar plus 0.005 gm. sugar inversion by 3 per cent. HCl for 15 min.).

TABLE X

ABSOLUTE RECOVERY OF INITIAL METABOLITES

Treatment: Number	Treatment: Conditions	Carbon recovered in discs + solution	Carbon evolved as CO_2	Volatile compounds	Total carbon recovered	Carbon removed as surface film on drying paper	Original dry weight of starch = carbon removed on drying paper	Starch previously accounted for‡	Total starch accounted for	Dry weight previously accounted for‡	Total dry weight accounted for	Original* calorific value of substance removed in drying paper	Calorific value previously accounted for‡	Total calorific value accounted for
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	kgm. cal.	kgm. cal.	kgm. cal.
1	Dist. H_2O, air	2.080†	0.185	0.014	2.279	0.249	0.561	3.762	4.323	5.900	6.461	2.37	24.61	26.98
2	0.005 equiv. KBr, air	2.073	0.193	0.014	2.280	0.248	0.558	3.704	4.262	5.793	6.351	2.355	23.70	25.95
3	0.05 equiv. KBr, air	2.033	0.228	0.017	2.278	0.250	0.562	3.763	4.325	5.190	6.472	2.375	23.66	26.03
4	0.005 equiv. KBr, 3.80 per cent. O_2	2.415	0.083	0.006	2.504	0.024	0.054	4.255	4.309	6.417	6.471	0.288	26.03	26.26
5	0.005 equiv. KNO_3, air	2.124	0.256	0.019	2.399	0.129	0.291	3.967	4.258	5.296	6.587	1.230	25.83	27.06
6	0.005 equiv. $CaBr_2$, air	2.235	0.170	0.013	2.418	0.110	0.247	4.154	4.401	6.304	6.551	1.04	25.94	26.98
7	Initial tissue	2.528	...	...	2.528	...	...	4.286	4.286	...	6.40	...	25.80	25.80

* Starch = carbon lost.
Starch = 4228 cal. per gram.

† Based on replicate analyses of one sample only, not mean of two parallel batches as in other cases.

‡ See table V.

TABLE XI

PERCENTAGE OF RECOVERY OF INITIAL METABOLITES

Treatment: Number	Treatment: Conditions	Recovery of original dry weight	Recovery of original starch	Recovery of original calorific value*
		%	%	%
1	Dist. H_2O, air	101.0	101.0	105.0
2	0.005 equiv. KBr, air	99.3	99.5	100.2
3	0.05 equiv. KBr, air	101.0	101.0	100.7
4	0.005 equiv. KBr, 3.80 per cent. O_2	101.0	101.0	102.0
5	0.005 equiv. KNO_3, air	103.0	100.7	104.8
6	0.005 equiv. $CaBr_2$, air	101.0	103.0	104.5

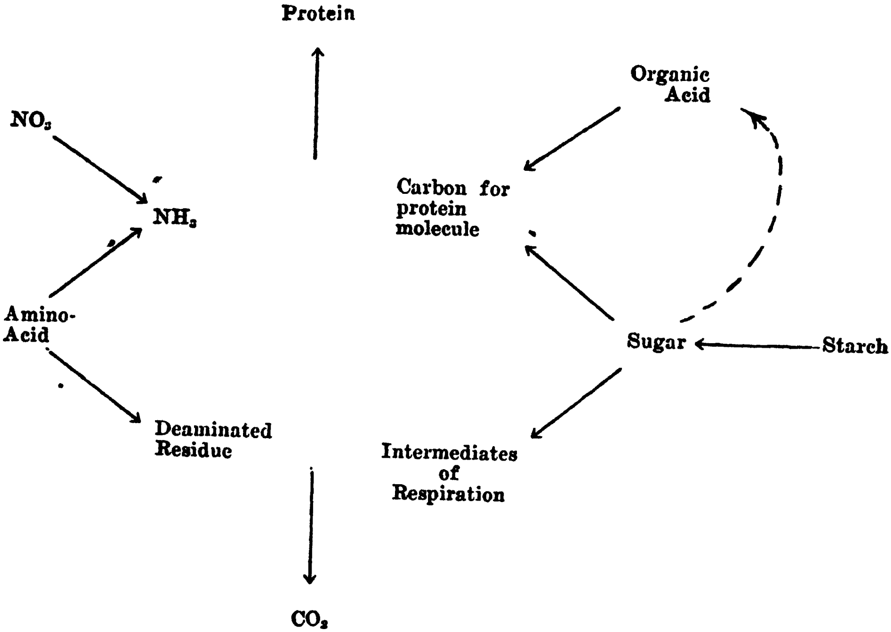
* Assuming all the heat of respiration $C_6H_{12}O_6 = 6CO_2 + 674{,}000$ cal. was absorbed by the surroundings.

Figure 1 implies that the same relationship exists between the nitrogen metabolism to protein and respiration whether the nitrogen is obtained direct from potassium nitrate or when the soluble nitrogen compounds of the cells are used. In the latter case the source of nitrogen also contributes carbon; in the former all the carbon involved is drawn from nitrogen-free reserves in the cells. The carbohydrate balance sheet (no. 5) is already complete although the nitrogen absorbed and converted to protein would require the carbon of approximately 3.5 per cent. of the total starch. Hence organic acids and not carbohydrate must be regarded as the probable source of carbon for the fabric of the protein molecule formed from nitrate and this strengthens the probability (see below) that these substances contribute the extra carbon necessary if synthesis proceeds from amino acids which are richer in nitrogen and poorer in carbon than the resultant protein.

There is, however, yet another apparent paradox. The connection between aerobic respiration and protein synthesis appears to be that deamination of amino acids liberates nitrogen-free residues and ammonia; the deaminated residues contribute to the carbon substrates of respiration and the ammonia forms protein with compounds derived in part, at least, from sugar and supplemented when necessary (*e.g.*, NO_3 culture no. 5) from organic acids. To preserve the appearance in the balance sheet that the carbohydrate carbon is accounted for apart from protein synthesis it is only necessary that the deaminated residues yield as much carbon dioxide as would otherwise be expected from that moiety of the protein molecule which is derived from sugar. Hence from the standpoint of the balance sheet the carbon content of the amino acids and the sugar which is built into the framework of the protein are interchangeable; it is immaterial which supplies the carbon for synthesis and which is converted to carbon

dioxide. It is clear, however that the *extra respiration stimulated by protein synthesis is more than could arise from the deaminated residues alone* and the excess must be due to an increased oxidation of sugar for which allowance has already been made in the carbohydrate balance sheet.

The relationships outlined above can be expressed as follows:



Though necessarily obscure as to details this scheme emphasizes that approximately ⅔ of the respiration of potato discs in water is attributable to a respiratory component which is intimately connected with protein synthesis and the processes of nitrogen metabolism. The pace of this intricate piece of metabolic machinery may be accelerated (*e.g.*, by oxygen supply, potassium, or nitrate ions) or depressed (by low oxygen concentration or calcium ions) by factors which ultimately affect all the component parts of a closely integrated system even though their immediate effects may be localized.

Other implications of the metabolic balance sheet of potato discs

In the presence of a calcium salt (no. 6) the balance sheet shows an apparent recovery of 103 per cent. of the original starch. The titration curve and pH data (47) and this paper (table I) show, however, that in the presence of a calcium salt the metabolism of organic acids was stimulated and the apparent recovery of more than the original starch in the balance sheet suggests that some of the carbon dioxide evolved came from

sources other than carbohydrate. In potato discs in calcium solutions protein synthesis and its associated phases of respiration are depressed and the indications are that the metabolism of organic acids makes a relatively greater contribution to the total carbon dioxide production.

Table V shows the effect on the calorific value of the removal of a surface film of organic matter from the discs by the blotting technique. Correcting for this the outstanding losses of calorific value can be compared with the heat value of respiration assuming that all of the carbon dioxide was produced aerobically from sugar. Assuming that the heat of respiration disappeared from the tissue system, an account of the original calorific value is rendered in table X. Treatments nos. 4 and 6 favored reactions (the relative preponderance of anaerobically produced carbon dioxide in case of no. 4 and the oxidation of organic acid in case of no. 6) which clearly have a lower energy value than that upon which the heat value of respiration was calculated; this is consistent with an apparent recovery of original calorific value somewhat greater than 100 per cent. Treatments nos. 2, 3, and 5, which stimulated aerobic respiration, provided the conditions most favorable to the uptake of ions, and in these cases the energy balance sheet most nearly approached to 100 per cent., assuming that *all of the energy of respiration was dissipated as heat and absorbed by the surroundings.* Even so, the apparent recovery of calorific value tends to exceed 100 per cent. but this does not mean that some of the energy of respiration was retained by the discs but rather that the heat value of respiration was assessed too highly since aerobic respiration includes some carbon dioxide which arises from reactions with a lower energy value.

The somewhat surprising possibility that all of the energy value of respiration is dissipated as heat and, under isothermal conditions, is absorbed by the surroundings is not without precedent. Both FIFE and ALGERA[13] (**47**), using organisms which in some respects are more tractable for the energy measurements (Azotobacter and Aspergillus respectively), found that the energy of respiration reappeared quantitatively as heat. In the data on calorific value of potato discs there is no evidence that the great decrease in their heat content which occurs during salt uptake (**41**) is directly applied to processes (*e.g.,* salt accumulation) in which work is done. At most the heat measurements record the change in the total heat content of the discs whereas the free energy change of the system determines its capacity to do work. Heat losses in the discs which are not due to respiration have been traced to causes which are *unconnected with the performance of osmotic work.* Present methods cannot measure the energy utilized in salt absorption *per se* which would represent only a very small part of the total energy turnover which occurs in the tissue.

[13] ALGERA produced an energy balance sheet in which the discrepancy was only 0.5 per cent. of the total—an amount within the experimental errors.

The fact of metabolic activity in storage tissue under conditions conducive to salt absorption is, however, conclusively established; this can be appreciated at once from figure 2. The magnitude of these effects may occasion some surprise even though previous papers (**38, 39, 40, 41, 42, 43, 44, 45**) anticipated that the relationship between salt uptake and vital processes would involve all the attributes of growing cells. Potato discs, though composed of storage tissue which has been supposed by many to be free of such complications, carry out all the processes (other than photosynthesis) of which parenchyma is capable. The specific "salt effects" are superimposed upon a basic condition of active metabolism—determined by the variables oxygen, temperature, and disc thickness—which is a prerequisite for salt accumulation (no. 1). Starch hydrolysis, rapid respiration, protein synthesis from amino acids, the disappearance of organic acids from the sap (**47**), an increased content of reducing substances (ascorbic acid and glutathione), and the activity of the catechol oxidase system of the potato (which also has the property of de-aminating amino acids), are all prominent features of this system in which salt accumulation may occur. Almost all of these processes are, however, affected by the nature and concentration of the salt in the external solution. The outstanding result is that all these processes which are stimulated by oxygen (compare treatments nos. 2 and 4) are intensified by the presence of potassium and still further by nitrate, whereas they are depressed by calcium. The effects due to the ions (K, NO_3, Ca) become intelligible when it is realized that they operate through the nitrogen metabolism.

The parallelism between aerobic respiration and protein synthesis revealed by figure 1 is a striking corollary to the demonstrable parallelism between aerobic respiration and salt accumulation (**21, 37**). Whereas the mechanism of deamination of amino acids provides a credible link between protein synthesis and respiration the connection between both processes and salt accumulation is still obscure. Schemata of respiration, which refer (**6**) to cells which have lost their capacity for constructive metabolism or are limited in their exercise of it by external variables, cannot be applied to the respiration of potato discs if they ignore the striking parallelism between the utilization of amino acids in protein synthesis and respiration which is so conspicuous a feature of the metabolism of these cells which are still able to grow and accumulate salts. There are several features of the schema described by GREGORY and SEN (**18**) which seem to apply to potato discs but these will be discussed in detail at a later date.

Specific reactions causally connected with salt accumulation remain elusive. Protein synthesis and aerobic respiration are intensified or depressed according as the nature of the salt, its concentration, or the oxygen conditions facilitate, or depress, absorption; and it is clear that these are

the aspects of metabolism which are most closely linked with salt uptake. The suggestion now is that water absorption is also brought about by metabolic processes in a manner not readily explained by any simple osmotic theory. As yet one can only conclude that, not one, but the summation of *all* the metabolic and biochemical processes described—processes dependent upon oxygen—constitutes that "dynamic machinery" which is essential for salt accumulation. The problem raised, however, is not only how these processes in living cells are directed to the actual accumulation of salts but *why they constitute such an apparently wasteful method of applying metabolic energy to the performance of useful work.*

Summary

The effects of six selected salt and oxygen treatments during 70 hours at 23° C. on the respiration and metabolism of potato discs have been investigated.

Those salt treatments (KNO_3, $CaBr_2$) in which uptake of the anion exceeds that of cation cause an increase in the bicarbonate concentration and alkalinity of the external solution.

In aerated solutions potassium salts stimulated, and calcium salts depressed, water absorption in a manner not wholly explicable by osmotic phenomena. This suggests that a re-investigation of water absorption and its relation to metabolic processes would be profitable.

At oxygen tensions such that respiration is not limited by oxygen the respiratory behavior of potato discs is determined by the salts in the external solution. When the concentration of potassium bromide in the external solution is increased, the respiration rate is also increased. In the presence of calcium bromide the respiration is less than in distilled water. At the same equivalent concentration potassium nitrate increases respiration more than does potassium bromide.

The effect of low oxygen concentrations on respiration has been confirmed.

All the treatments cause an increased sugar concentration relative to that in the initial tissue. The salt and oxygen treatments which stimulate respiration produce a low, residual sugar concentration. The concentration of sugar does not regulate the respiration rate.

At a low oxygen concentration (solution in equilibrium with 3.80 per cent. O_2) at which accumulation of bromide was depressed, synthesis of protein from amino acid was also limited by oxygen lack. In aerated distilled water protein synthesis occurs and this is increased by the presence in the external solution of potassium bromide but is decreased by calcium bromide. Dilute potassium nitrate produced the greatest observed effect on protein synthesis.

An approximately linear relationship was found between the amount of protein synthesized and the quantity of carbon dioxide respired.

Approximately ⅓ of the respiration of discs in aerated distilled water is produced independently of nitrogen metabolism. This component of the total respiration is not affected by salts or oxygen concentration. Two thirds of the respiration of discs in distilled water arises from a component of respiration which is linked to protein synthesis from amino acids, is affected by salts and oxygen concentration and in which the oxidase system is involved.

The effects of inorganic ions on respiration are parallel to their effect on protein synthesis. Potassium and nitrate ions intensify, while calcium retards, the effect of oxygen on respiration.

The characteristic effects of ions on respiration and synthesis are exerted at oxygen tensions at which their absorption occurs and the reactions of the ions observed therefore constitute an integral part of the machinery of salt absorption.

A preliminary balance sheet of metabolites showed that significant amounts of carbon, dry weight, carbohydrate, and calorific value were not accounted for when the tissue respired in aerated water or potassium bromide solution.

The discrepancies in the balance sheet proved to be caused by the transfer of organic substance from the surface of those discs which had been in aerated potassium bromide solution or distilled water to the drying papers used to remove surface water. The losses named were real at high oxygen tensions, did not occur at low oxygen tension, and were small in the presence of calcium salts.

The surface film removed was formed at the expense of the starch fraction and consisted of a complex rich in uronic acid. If the uronic acid removed from the discs by blotting is expressed as pectin the concomitant loss of carbon can be accounted for and also a large part of the loss of dry weight.

It is now believed that losses of magnesium referred to in an earlier paper (**38**) are attributable to a similar cause.

Volatile organic compounds are produced by potato tissue under the conditions of these experiments only in small amount. Such compounds appear in the flowing gas stream and small amounts are evolved when the tissue is dried. The carbon value of the total production of volatile compounds is of the order of 7.5 per cent. of the total respiration.

A revised balance sheet embodies corrections for all the above processes and summarizes satisfactorily the principal processes which occur in potato discs under the conditions conducive to salt uptake and the effects of salts and oxygen upon them at 23° C.

The amino acids and sugar supply the carbon for the protein molecule and the extra respiration entailed in its production. In the fixation of nitrate nitrogen, carbon is derived from other sources (organic acids). Organic acids disappear during protein synthesis from amino acids and probably contribute here also to the carbon framework of the protein molecule.

Experimental difficulties prevent an estimate of the energy involved in salt uptake *per se*. The implication of the data is that all of the energy liberated by respiration is absorbed by the surroundings as heat.

The significance of the results relative to current views on respiration and salt uptake is discussed.

The work here described represents the results of an investigation started in the Division of Plant Nutrition, University of California, in 1933–1934. During this period the senior author received leave of absence from the University of Leeds for which he is indebted to PROFESSOR J. H. PRIESTLEY. The work has been continued since 1934 in both the laboratories mentioned. For the continued help supplied by the Division of Plant Nutrition we wish to express our thanks and also for the personal contribution of PROFESSOR D. R. HOAGLAND to our work. It should be recognized that after the preliminary stages DR. P. R. STOUT, a member of the Division of Plant Nutrition of the University of California, was almost solely responsible for the calorimetric work and many of the quantitative determinations in this paper whereas DR. C. PRESTON contributed the experimental data dealing with volatile compounds and the loss of dry matter by blotting the discs. For the determinations of the nitrogen and carbohydrate fractions we have to thank Dr. W. Z. HASSID of the Division of Plant Nutrition.

BIRKBECK COLLEGE
UNIVERSITY OF LONDON, AND
THE UNIVERSITY OF CALIFORNIA

LITERATURE CITED

1. ARCHBOLD, H. K., and BARTER, A. M. The relation of carbon dioxide output to the loss of sugar and acid in Bramley's seedling apples during storage. Ann. Bot. **48**: 957–966. 1934.
2. BAPTISTE, E. C. D. The effect of some cations on the permeability of cells to water. Ann. Bot. **49**: 345–366. 1935.
3. BARKER, J. Analytical studies in plant respiration. IV and V. The relation of the respiration in potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Proc. Roy. Soc. B. **112**: 316–358. 1933.
4. ——————. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. Proc. Roy. Soc. B. **119**: 453–473. 1936.

5. BENNET-CLARK, J. A., GREENWOOD, A. D., and BARKER, J. W. Water relations and osmotic pressures of plant cells. New Phytol. **35**: 277–291. 1936.
6. BLACKMAN, F. F. Analytical studies in plant respiration. III. Formulation of a catalytic system for the respiration of apples and its relation to oxygen. Proc. Roy. Soc. B. **103**: 491–523. 1928.
7. BONNER, J. Chemistry and physiology of the pectins. Bot. Rev. **10**: 475–497. 1936.
8. BOSWELL, J. G., and WHITING, G. C. A study of the polyphenol oxidase system in potato tubers. Ann. Bot., N.S. **2**: 847–864. 1938.
9. DAMODARAN, M., and NAIR, K. R. A glutamic acid dehydrogenase from germinating seeds. Biochem. Jour. **32**: 1064–1074. 1937.
10. DASTUR, R. H., and DESAI, M. The carbon dioxide carbohydrate ratio in the aerobic and the anaerobic respiration of rice. Ann. Bot. **49**: 53–69. 1935.
11. DENNY, F. E., and MILLER, L. P. Production of ethylene by plant tissue as indicated by the epinastic response of leaves. Contrib. Boyce Thompson Inst. **7**: 97–102. 1935.
12. DORE, W. H. The composition of pectin: A preliminary report of the determination of galacturonic acid in pectin. Jour. Amer. Chem. Soc. **48**: 232–236. 1926.
13. ————. The pectic substances. Jour. Chem. Educ. **3**: 505–513. 1926.
14. ERNST, E. Osmosis in physics and biology. Nature **141**: 80. 1938.
15. FISHER, R. A. Statistical methods for research workers. London. 1932.
16. GANE, R. Combustible gaseous products of fruits. Rep. Food Invest. Board, Sec. V: 122–130. 1934.
17. ————. The formation of ethylene by plant tissues and its significance in the ripening of fruits. Jour. Pomol. **13**: 351. 1935.
18. GREGORY, F. G., and SEN, P. K. Physiological studies in plant nutrition. Ann. Bot. N.S. **1**: 521–561. 1937.
19. HAMNER, K. C. Effects of nitrogen supply on the rates of photosynthesis and respiration in plants. Bot. Gaz. **97**: 744–764. 1936.
20. HAPPOLD, F. C., and RAPER, H. S. The Tyrosinase-tyrosine reaction. The supposed deaminising action of tyrosinase on amino-acids. Biochem. Jour. **19**: 91–100. 1925.
21. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
22. HOPKINS, E. F. Variation in sugar content in potato tubers caused by

wounding and its possible relation to respiration. Bot. Gaz. **84**: 75–88. 1927.

23. LUNDEGÅRDH, H. Untersuchungen über Anionenatmung. Biochem. Zeitschr. **290**: 104–124. 1937.
24. ————, and BURSTRÖM, H. Untersuchungen über die Salzaufnahme der Pflanzen. Biochem. Zeitschr. **261**: 235–251. 1933.
25. NIGHTINGALE, G. T. The nitrogen nutrition of green plants. Bot. Rev. **3**: 85–174. 1937.
26. PEARSALL, W. H., and EWING, J. The absorption of water by plant tissue in relation to external hydrogen ion concentration. Jour. Exp. Biol. **4**: 245–257. 1927.
27. ————, and ————. The relation of nitrogen metabolism to plant succulence. Ann. Bot. **43**: 27–33. 1929.
28. ————, and BILLIMORIA, M. C. Losses of nitrogen from green plants. Biochem. Jour. **31**: 1743–1750. 1937.
29. PETRIE, A. H. K., and WOOD, J. G. Studies on the nitrogen metabolism of plants. I. The relation between the content of proteins, amino-acids and water in the leaves. Ann. Bot., N.S. **2**: 33–60. 1938.
30. PRIESTLEY, J. H., and WOFFENDEN, L. M. The healing of wounds in potato tubers and their propagation by cut sets. Ann. App. Biol. **10**: 96–115. 1923.
31. RAISTRICK, H. *et al.* Studies in the biochemistry of microorganisms. Phil. Trans. Roy. Soc. London B. **220**: 1–367. 1931.
32. RAPER, H. S. The aerobic oxidases. Physiol. Rev. **8**: 245–282. 1928.
33. RICHARDS, F. J. Physiological studies in plant nutrition. VIII. The relation of respiration rate to carbohydrate and nitrogen metabolism of the barley leaf as determined by phosphorus and potassium supply. Ann. Bot., N.S. **2**: 491–534. 1938.
34. REINDERS, D. E. The process of water-intake by discs of potato tuber tissue. Kon. Neder. Akad. v. Wetensch. **41**: 1–14. 1938.
35. ROBBINS, W. J. The isoelectric point for plant tissue and its importance in absorption and toxicity. Univ. Missouri Studies **1**: 3–60. 1926.
36. ————. The analogy between plant tissue and a protein. Proc. Int. Congress Plant Sciences **2**: 1125–1138. 1929.
37. STEWARD, F. C. On the evidence for phosphatides in the external surface of the plant protoplast. Biochem. Jour. **22**: 268–275. 1928.
38. ————. The absorption and accumulation of solutes by living plant cells. I. Experimental conditions which determine salt absorption by storage tissue. Protoplasma **15**: 29–58. 1932.
39. ————. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.

40. ————. V. Observations upon the effects of time, oxygen and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.

41. ————. Mechanism of salt absorption by plant cells. Nature **135**: 533. 1935.

42. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **4**: 521–544. 1935.

43. ————. Salt accumulation by plants—the rôle of growth and metabolism. Trans. Far. Soc. **33**: 1006–1016. 1937.

44. ————, Berry, W. E., and Broyer, T. C. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 345–366. 1936.

45. ————, Wright, R., and Berry, W. E. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water with observations upon surface volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.

46. ————, and Harrison, J. A. The absorption and accumulation of solutes by living plant cells. IX. The absorption of rubidium and bromide by potato discs. Ann. Bot. N.S. **3**: 427–454. 1939.

47. ————, and Preston, C. Metabolic processes of potato discs under conditions conducive to salt accumulation. Plant Physiol. **15**: 23–61. 1940.

48. Stiles, W. Studies on toxic action. Protoplasma **11**: 349–381. 1930.

49. ————, and Jorgensen, I. Studies in permeability. V. The swelling of plant tissue in water and its relation to temperature and various dissolved substances. Ann. Bot. **31**: 415–434. 1917.

50. ————, and ————. On the relation of plasmolysis to the shrinkage of plant tissue in salt solutions. New Phytol. **18**: 40–49. 1919.

51. Thoday, D. Some observations on the behavior of turgescent tissue in solutions of cane sugar and of certain toxic substances. New Phytol. **17**: 57–68. 1918.

EFFECT OF SOIL MOISTURE ON GROWTH AND TRANSPIRATION IN *HELIANTHUS ANNUUS*

EMMETT V. MARTIN

(WITH FOUR FIGURES)

Introduction

Work done previous to 1913 concerning the effects of soil moisture on growth and transpiration of plants is summarized in a paper by BRIGGS and SHANTZ (2). Practically all of these earlier workers found that there was an increase in growth with increasing holard over most of its range, although growth was somewhat reduced in soil that was nearly or completely saturated. Water requirement usually increased regularly with holard, but in a few cases very low values of soil moisture gave higher water requirements.

TUMANOW (16) has grown sunflowers in small containers holding six kilograms of dry soil. The holard of one set of plants was maintained near 60 per cent. of the maximum capacity of the soil, while that of another set was allowed to vary between this value and the wilting point. The experiment was continued throughout the life cycle, during which time the test plants experienced sixteen wiltings. At the end of the series the control plants had about twice the dry weight of the others, but only 20 per cent. greater water requirement.

TUMANOW also grew bean plants to flowering in pots containing 3500 gm. of dry soil. One set (the control) was maintained at a holard of 60 per cent. of the capacity of the soil, while the other two sets were started with holards of 50 per cent. and 40 per cent., but neither of the latter was given any additional water during the growing period. At the end of the series, the average holards of these last two groups were 24 and 30 per cent. respectively. The dry weights were 11.66 grams for the control set, 5.43 for the 50 per cent. group, and 2.21 for the 40 per cent., while the water requirements were 136, 108, and 86 respectively.

CLEMENTS and LONG (3, 4) grew sunflowers in metal containers eight inches in diameter and ten inches tall with average holards maintained approximately at 14, 18, 26, and 35 per cent. of the dry weight of the soil, and found that growth and water requirement increased regularly with holard. This was true in the sun as well as in the shade, although in low light values the influence of holard on the water requirement was more marked.

PESSIN (14) grew seedlings of *Pinus palustris* and *Pinus caribaea* for six months in quart containers with holards equal to 5, 10, 15, 25, and 35 per cent. of the dry weight of the soil. The dry weights of both species

increased with holard to 25 per cent., but those in 35 per cent. were smaller. The water requirement of *Pinus caribaea* rose regularly from 364 for the 5 per cent. group to 1163 for the 35 per cent., while that of *Pinus palustris* had a maximum at 15 per cent. holard. In neither species was there any evidence of an increase for the very low values of available water.

BRIGGS and SHANTZ (2) and SHANTZ (15) have raised the objection to much of the earlier work on the effects of different degrees of soil moisture on growth and transpiration that it is impossible to maintain a mass of soil at a uniform low holard, because the small amounts of water added from time to time are insufficient to moisten the entire mass of soil.

VEIHMEYER (17) has done a great deal of work relating to the penetration of water into soil, and has found that water applied to the surface of soil penetrates only to such a depth that the soil through which it passes is raised to its field capacity. Further penetration is exceedingly slow, and ordinarily is of little consequence to plants. If a plant growing in a container has reduced the holard to the wilting point, the only way in which the water-content of the entire mass of soil can be raised is to add enough water to bring all of the soil to its field capacity. Addition of less than this amount will leave the holard of some of the soil in the bottom of the container unchanged. In such a case, the plant would be forced to grow in a smaller volume of soil than another plant in a similar container with higher average holard.

In conducting studies of the effect of soil moisture on plants, VEIHMEYER (17) recommends avoiding this difficulty of soil-water distribution by adding at each irrigation enough water to bring all the soil to its field capacity, but allowing different plants to reduce the holard to different degrees of dryness before again adding water. This procedure permits each plant to have water available throughout the entire soil mass, but the various plants would be working through different ranges of soil moisture. Using this method with prune trees, he has found that growth and rate of extraction of water from the soil are not appreciably affected over the range of holard from the field capacity to approximately the wilting percentage, results which are not at all in accordance with previous work.

HENDRICKSON and VEIHMEYER (5, 6, 7, 8), experimenting in the field with prune, peach, pear, and walnut trees and grape vines, have obtained similar results, and have arrived at the conclusion (5) that "trees either *have* readily available water or *have not.*" BECKETT, BLANEY, and TAYLOR (1), working in citrus and avocado orchards, arrived at a similar conclusion.

MAGNESS, DEGMAN, and FURR (13) have found that the behavior of stomata of apple trees may be affected when the moisture content of the whole root zone is apparently considerably above the wilting percentage, while at least part of the root zone on soils of medium or light texture is

usually at or near the wilting percentage before variations in the growth rate of the fruit can be detected.

Very different results have been obtained by LEWIS, WORK, and ALDRICH (**9, 10**) and by WORK and LEWIS (**19**) with pears on a heavy clay soil. They found that the fruit growth rate was reduced whenever the soil moisture was lowered below 70 per cent. of the available capacity.

An explanation of these apparently contradictory results has been advanced by MAGNESS (**12**) and by LEWIS, WORK, and ALDRICH (**10**). They suggest that, in those cases in which trees have suffered from water-shortage when the soil moisture was well above the wilting percentage, the trees were growing on heavy soils with slow capillary movement of water and poorly distributed root systems. As a result, the soil in immediate contact with the absorbing roots may be at or near the wilting percentage while the average in masses large enough to be sampled may be well above this point. On the other hand, experiments which have shown that soil moisture is equally available from the field capacity to about the wilting percentage have been with trees growing on moderate to light textured soils in which the root distribution is usually much more complete and capillary movement more rapid than in heavy soils. According to this hypothesis, in all cases of water-shortage the soil moisture in the immediate vicinity of the absorbing roots is at or near the wilting percentage, even though only a short distance away it may be well above this point. Failure of the tree to obtain water under these circumstances is presumed to be due to the relatively great spacing of the roots and slow capillary movement of water through the soil.

The present investigations were undertaken with the purpose of obtaining further information concerning the effect of soil moisture on growth and transpiration of *Helianthus annuus*, using a light, fertile, sandy loam in order to permit as great a degree of root growth and capillary movement of water as possible.

Methods

For all experiments reported in this paper, plants of the Russian Mammoth variety of *Helianthus annuus* obtained from commercially produced seed were used. The seeds were soaked in water 24 hours before being planted in special pots made of asphalt felt (tar paper) rolled into a cylinder about 2 inches in diameter and 8 inches long. With this type of pot, it was possible to transfer the seedlings to the phytometer cans without disturbing the roots. Four seeds were planted in each pot, and the seedlings were selected for uniformity at the time the cans were sealed.

For all series, galvanized iron cans 13 inches in diameter and 22 inches tall with removable lids were used. The soil employed was a rather light, sandy loam with a field capacity of 18 per cent. and a permanent wilting

coefficient of 7 per cent. of the dry weight. All the soil used in any particular series was thoroughly mixed as a whole in order to have as great a degree of uniformity among the cans as possible.

For series I–III, the soil for each can was weighed out and mixed in a metal pan with about 100 grams of the commercial fertilizer Vigoro. It was finally run through a 6 × 6 mesh screen and packed firmly in the cans. The seedlings were then transplanted and enough water was added to bring the soil to its field capacity. As soon as the seedlings were tall enough to project above the lid, the opening in the center of the lid was sealed around the stem of the plant with modelling clay. This seal excluded rain and reduced evaporation satisfactorily. For each of these three series, 12 cans were used. They were divided into two sets of 6 each and given different treatments as follows: one group, called the control, was watered frequently in order to keep the holard above 14 per cent. The other group, called the test set, was watered only when the holard had been reduced to about 10 per cent., and then was given enough water to bring the soil back to its field capacity. All water was added to the surface of the soil. Samples indicated that at least 12 hours were required for water to penetrate to the bottom of the can, so that ordinarily watering was done in the late afternoon in order to allow penetration during the night when transpiration was low. On this account, it was necessary to discontinue these series when the control plants were nearly to the point of needing water more than once each day.

For series IV–V, the procedure was different in that the soil was mixed at a predetermined holard at the time of filling the cans, and no water was added at any time during the series. Four groups of 6 cans each were employed, with average initial holards of approximately 11, 14, 17, and 20 per cent. of the dry weight of the soil. The soil for each can was mixed in a flat metal pan with about 100 grams of Vigoro and enough water to bring the soil to the desired holard. The soil was finally run through a 6 × 6 mesh screen and pressed down firmly in the can. For the group with 20 per cent. holard, the soil was mixed at 14 per cent. and was brought to its proper value by the addition of water after it was placed in the cans. The seedlings were transferred to the cans immediately after filling. A circular piece of tar paper cut to fit the inside of the can was placed on top of the soil. This paper had a 2-inch circular hole in the center for the plant. Both this hole and the gap around the edge of the paper were sealed with modelling clay. The lid of the can was put on at this time, and as soon as the plant projected through, the hole in the lid also was sealed around the stem of the plant with clay as in the other series. This seal was very effective in controlling evaporation from the soil and in excluding rain. Cotton placed around the stem of the plant prevented the seal from getting

too hot due to exposure to radiation. The cans were weighed once each week on a platform scale having a sensitivity of 1/100 pound. Both these series were brought to a close when the holard of the 11 per cent. group had been reduced to about 9 per cent.

At various times throughout the series, measurements of stem height, stem diameter, and leaf area were made. The leaf areas in square centimeters were determined by multiplying the products of length and width of the leaves by the factor 1.34. This procedure yielded the sum of the areas of both sides of the leaf. At the end of a series, final measurements were taken and the plants were dried in an electric oven at 65° C. The roots were washed out of the soil by a stream of water from a nozzle.

Measurements of the size and degree of opening of the stomata on the lower surface of the leaves were made from strips of epidermis preserved in absolute alcohol according to the method of LLOYD (**11**).

Values of soil moisture during the series were calculated from the weights of the cans and the initial known holard, although occasional samples were taken in order to correct for the changing weight of the plant. For this purpose, a circular hole about 4 inches in diameter was dug with a trowel near the edge of the can, and samples of approximately 200 grams each were taken from the inner side of the hole at depths of 2, 8, 14, and 20 inches from the top surface of the soil. This procedure gave samples at points about midway between the center and edge of the soil mass. This method of sampling disturbed the root system some, but there was no apparent effect on the shoot, either in appearance or in growth rate. No can was sampled more than once. Roots were screened out of the sample before drying.

The validity of the average of a group of plants depends upon having all individuals of a group in the same environment. In the case of soil moisture studies, all plants of a given group should be growing in soil with the same holard. On account of the fact that different plants use water at different rates, this condition is possible only within certain limits. In the experiments reported in this paper, the cases were very rare in which the average holard for any plant deviated more than 1 per cent. from the average of its group, and many of them were within 0.5 per cent. These limits seemed to be sufficiently narrow to justify averaging the results of the individuals in the various groups.

Results

SERIES I–III

The values of holard, leaf area, and transpiration rate per unit leaf surface for series II and III are shown graphically in figure 1. As the graph shows, there was a noticeable difference in the leaf areas of the test and

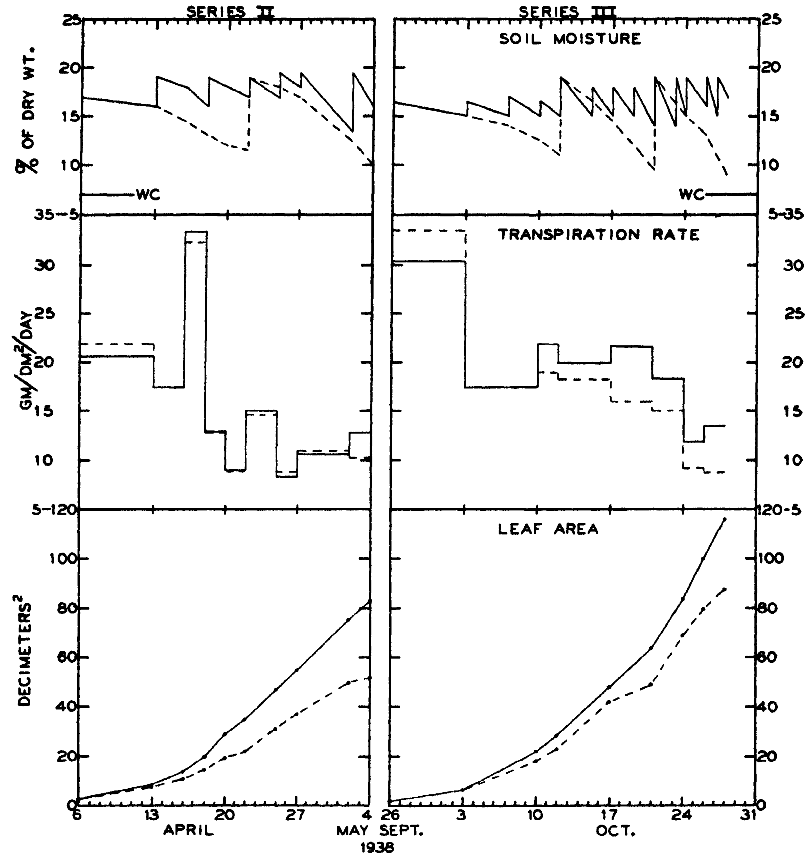


FIG. 1. Variation in soil moisture, transpiration rate, and leaf area during series II and III. Values for the control and test groups are indicated by the solid and broken lines respectively.

control plants by the time the average holard of the former had been reduced to 13 per cent., and the difference was markéd at 11 per cent., when the test set was given its first watering. At this point in series II, the control group had a leaf area 58 per cent. greater than that of the test set, while for all three series the average difference was 41 per cent. In all three series the differentiation began almost as soon as the test set was given its first watering.

It was possible that, during the period immediately following this watering of the control set, the plants of the test set might be using the water only from the soil in the upper part of the cans, reducing this portion to near the wilting coefficient but leaving the lower part moist, thus producing the observed difference in growth. In order to obtain some information

on this point, soil samples were taken in series II on April 22, just before watering the test set. The holard at depths of 2, 8, 14, and 20 inches in the test set was found to be 10.1, 10.6, 11.1, and 10.8 per cent., respectively. On Oct. 10 in series III, samples at the same depths showed holards in the test set of 11.1, 11.6, 13.4, and 11.9 per cent. Although this does not demonstrate that the soil immediately in contact with the roots was not at or near the wilting coefficient, it does show that these plants removed the moisture from all regions of the soil in a remarkably uniform manner.

The increase in leaf area of the plants in series II and III during consecutive intervals of the growing period in percentage of the values at the beginning of the intervals is given in table I. In both series, as the holard

TABLE I

INCREASE IN LEAF AREA OF THE PLANTS IN SERIES II AND III DURING CONSECUTIVE INTERVALS OF THE GROWING PERIOD IN PERCENTAGE OF THE VALUE AT THE BEGINNING OF THE INTERVAL IN QUESTION

SERIES II

INTERVAL	APR. 6 APR. 13	APR. 13 APR. 16	APR. 16 APR. 18	APR. 18 APR. 20	APR. 20 APR. 22	APR. 22 APR. 25	APR. 25 APR. 27	APR. 27 MAY 2	MAY 2 MAY 4
Control	233	54.5	43.9	46.5	18.8	35.1	16.4	37.6	10.0
Test	218	39.2	34.6	32.4	12.8	41.2	17.9	36.4	2.6

SERIES III

INTERVAL	OCT. 3 OCT. 10	OCT. 10 OCT. 12	OCT. 12 OCT. 17	OCT. 17 OCT. 21	OCT. 21 OCT. 24	OCT. 24 OCT. 26	OCT. 26 OCT. 28
Control	224	30.9	68.6	33.6	30.8	19.5	16.0
Test	177	24.2	82.4	16.4	40.2	16.0	9.9

of the test set decreased, the rate of increase of leaf area of this group dropped off with respect to that of the control; for periods immediately after watering, the rate of increase rose above that of the control. For periods just previous to watering, the rate of growth of the test group (as measured by leaf area increase) varied from about one-half to three-fourths that of the control; afterward the rate rose to about 1.2 times the latter. This same behavior was noted in stem diameter, although it was less marked and less regular, presumably on account of less accurate measurements. This effect is probably due largely to recovery of turgor, although it is possible that there is an increase in the rate of assimilation, as TUMANOW (**16**) claims.

The rate of transpiration per unit of leaf surface was not entirely consistent in its relation to holard. In series II the rate was not affected until the holard had dropped below 11 per cent., but in series III the effect was noticed at about 12 per cent. This difference is slight, and is probably due to the somewhat higher transpiration rates in the latter case. In series III, after the initial reduction in transpiration rate with low holard, the test

plants never recovered equality with the controls. This effect was not evident in series II, since the first period of low holard did not affect the transpiration rate. In series I, the rate of transpiration was reduced about 20 per cent. at a holard of 11 per cent., but it regained equality with the controls after watering. TUMANOW (16) found that his sunflower plants after recovery from wilting had a transpiration rate well above that of the controls.

Measurements of the diurnal variation of stomatal opening on several occasions during series II gave the following results. On April 21, a time of relatively low holard of the test set, the stomata of the test set closed partially about 2:00 P.M. while those of the control plants remained open. On April 23 and 27, times of high holard and both cloudy days, stomata remained open all day on both groups. On May 3, at low holard of the test set, stomata remained nearly closed all day on the test set and practically wide open on the control. This was the only period during which the transpiration rate of the test set was appreciably reduced below that of the control. It was also the only period during which the test plants showed any visible signs of flaccidity of the leaves, although even in this case the wilting was very slight.

For each of the three series, the plants were allowed to grow until those

TABLE II

SUMMARY OF DATA OBTAINED FROM SERIES I–III. ALL MEASUREMENTS ARE AVERAGES OF ALL PLANTS IN EACH GROUP

ITEM	SERIES I		SERIES II		SERIES III	
	TEST GROUP	CONTROL GROUP	TEST GROUP	CONTROL GROUP	TEST GROUP	CONTROL GROUP
Number of plants in group	4.0	5.0	6.0	6.0	6.0	6.0
Stem height (cm.)	75.0	87.0	68.0	65.0	73.0	83.0
Stem diameter (cm.)	2.0	2.33	1.89	2.30	1.85	2.48
Leaf area (dm.²)	63.3	101.0	51.5	82.8	87.6	116.0
Fresh weight of shoot (gm.)	413.0	654.0	320.0	511.0	424.0	688.0
Dry weight of shoot (gm.)	49.4	70.8	37.4	53.1	53.0	73.5
Dry weight of roots (gm.)	11.4	12.3	9.82	14.2	11.5	13.5
Dry weight, total (gm.)	60.8	83.1	47.2	67.3	64.5	86.9
Shoot/root ratio	4.53	5.97	3.83	3.93	4.76	5.48
Percentage of water in shoot	88.0	89.2	88.3	89.6	87.5	89.4
Total transpiration (kg.)	11.49	18.88	10.97	16.16	15.39	22.70
Water requirement (gm./gm.)	189.0	227.0	235.0	240.0	239.0	267.0
Number of stomata per mm.²	219.0	153.0	256.0	213.0	367.0	344.0
Thickness of leaf (microns)	....	....	249.0	316.0	221.0	246.0
Palisade (percentage of total thickness)	....	....	49.0	50.0	55.0	55.0
Date of planting seeds	Jan. 15, 1938		Feb. 24, 1938		Sept. 8, 1938	
Date of harvesting plants	April 13. 1938		May 6, 1938		Oct. 28, 1938	

in the control group were nearly to the point of needing watering more than once each day. At this time the series were discontinued and final measurements taken. The results are given in table II, and those from series III are shown in figure 2. For all three series, the control plants

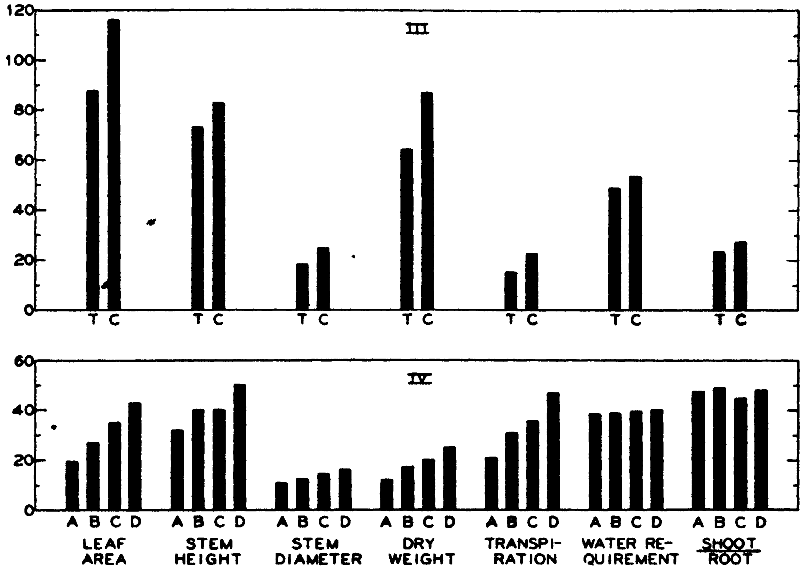


FIG. 2. Final measurements taken in series III and IV. The ordinate represents leaf area in square decimeters, stem height in centimeters, stem diameter in millimeters, dry weight in grams, transpiration in kilograms for series III and hectograms for series IV, water requirement in grams per gram × ⅕, and shoot-root ratio × 5.

had the greatest stem diameter, leaf area, dry weight of shoot and roots, shoot-root ratio, percentage water in shoot, total transpiration, and water requirement.

Statistical tests according to the method of "Student," as outlined by FISHER, show that for all three series the differences in growth in terms of stem diameter, leaf area, and total dry weight are all significant (odds greater than 99:1). Similar results were obtained for water requirement in series I and III, but not in II. The odds are only about 5:1 that differences in stem height and shoot-root ratio in series I and III are real, but in II they are still less.

Series II appeared to be different from the others in that the shoot-root ratio, transpiration rate per unit leaf area, stem height, and water requirement were affected little if any by the different treatments. It might be noted in this connection that in the two series in which the transpiration rate per unit area was markedly affected, the water requirement was also reduced considerably, while in the other series neither was altered much.

Leaf sections were made in series II and III for measurements of the thickness of the leaf and the palisade tissue. In both series, the thickness of the leaves of the control plants was greater than that of the test plants, but the proportion of the leaf composed of palisade was the same for both sets. The epidermal cells of the test plants were slightly smaller than those of the control. Stomata were more numerous and smaller in the test plants, the size being in inverse proportion to the frequency. The anatomy of the leaf appeared to be largely a case of failure to expand under the reduced water-content of the soil, presumably due to the lower turgor of the leaves.

Short-period series

At the time of starting the plants of series III, six extra plants were put in cans of the same size and were treated like the controls throughout their growing period. Near the end of series III, these six plants were used for a short-period series. They were standardized by determining the transpiration rates per unit area for one day, and two sets of two plants each were selected such that the average rates of the two groups for that day were equal. These two groups were then considered to be a test and a control group, and for about ten days were treated in the same manner as the other plants in series III had been earlier. The variation of holard, leaf area, and ratio of transpiration rates of the test and control plants during the ten-day period is shown in figure 3.

The first portion of the transpiration curve shows considerable fluctuation about the ratio unity, but statistical tests show that this much variability is to be expected when only two plants are used in each set. Under these conditions, differences of about 20 per cent. in the transpiration rate per unit area are ordinarily detectable with two plants per set.

The ratio of the transpiration rate of the test set to that of the control was not appreciably altered until the holard of the former had been reduced to approximately 11 per cent.; at this point the ratio was about 0.8. Below this value of holard, the ratio dropped rapidly, reaching a value of 0.5 at 8 per cent. After the test set was watered, its rate of transpiration rose to equality with that of the control. The ratio was maintained above 0.8 until the holard of the test set had again dropped to 10 per cent., but it was reduced to 0.5 at 8 per cent. As before, watering led to a resumption of normal transpiration rate. Tumanow (16) reported an increase in the transpiration rate of sunflower plants after recovery from wilting above that of plants which have not wilted, but no such effect was noted in the experiments reported here.

Stomata were examined at each time of weighing the plants, and the degree of opening was found to be affected by holard only when the transpiration rate was reduced. Through the first period of falling transpira-

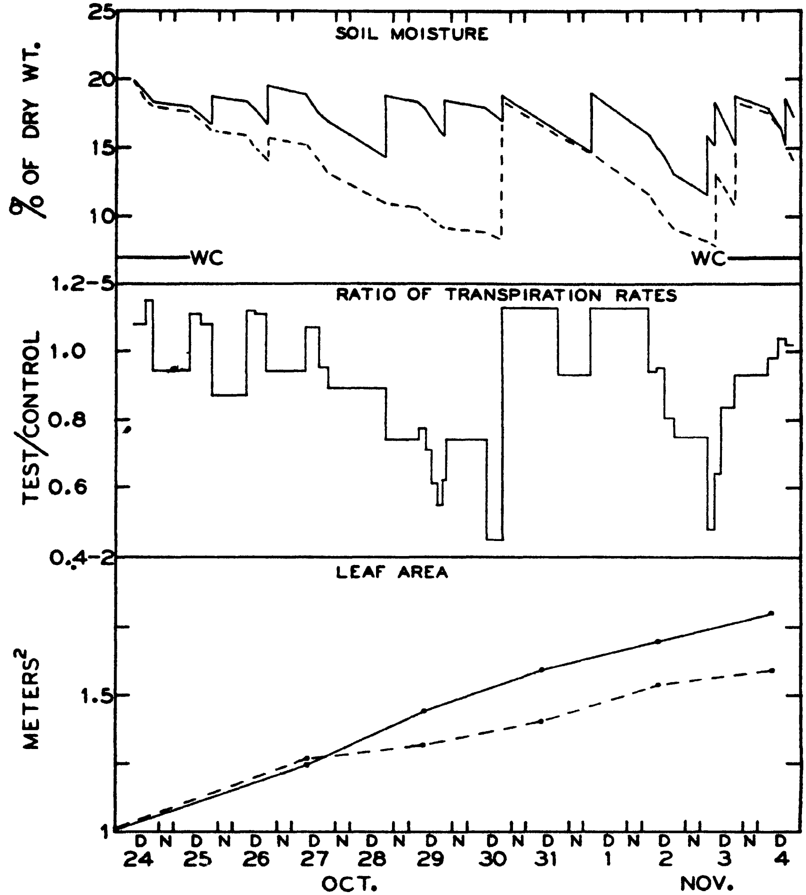


FIG. 3. Variation in soil moisture, ratio of transpiration rates of test and control groups, and leaf area during the short-period series.

tion rate, the stomatal opening of the plants of the test set became smaller and smaller until it appeared to be practically zero on Oct. 30, when the ratio was about 0.5. Since the plants were watered that evening, the stomata behaved the next day like those of the control set. At about noon on Nov. 2, when the transpiration ratio had again reached 0.8, the stomata of the test set began to close. They failed to open to more than mere slits the next morning. The plants were watered at 10:00 A.M. on this day; by noon the stomata were beginning to open, and at 4:00 P.M. they were as wide open as normally. In every case of reduced transpiration rate and closure of stomata there was a noticeable flaccidity of the leaves. This effect was slight at a transpiration ratio of 0.8, but there was marked wilting at a ratio of 0.5.

As in the other series above, these plants show an apparent increase in the rate of leaf growth after recovery from reduced turgor. For the period Oct. 29–31, the increase in leaf area of the control set was 10.4 per cent., while that of the test set was 6.9 per cent. From Oct. 31 to Nov. 2, after watering the test set, the values were 6.6 and 9.6 per cent., respectively. This difference is very probably not due to errors in measurement of leaf area, since comparisons of this method with that of measuring the areas of blueprints with a planimeter indicate that the probable error in the mean of the leaf areas of two plants of the size employed in this series is about 1.5 per cent. Since this degree of error appears to be caused largely by variations in the shape of leaves on different plants, it is probable that measurements of increases in leaf area for the same plants can be made with still greater accuracy.

One possible explanation of this phenomenon is that there is an increase in the rate of assimilation of plants recovered from wilting with consequent increase in rate of expansion of leaves. TUMANOW (16) presents data which indicate that such is the case, although the data are not convincing.

A more logical hypothesis is that more than 18 hours were required in this case for complete recovery of turgor of the leaves, although sunflower plants ordinarily apparently recover turgor within two or three hours after watering. Since this explanation cannot be ruled out, the data cannot be taken as clear evidence of anything more than this effect.

SERIES IV–V

The variation of holard, leaf area, and transpiration rate per unit of leaf surface during series IV is shown in figure 4. For the first week of the series, the transpiration rate of set A was higher than that of the other sets; however, statistical tests indicate that the differences are not significant. The same relation held for the second week, but for succeeding periods the rate of the sets with more water was greater than that of those with less. Numerous statistical tests show that differences in transpiration rate of approximately 15 per cent. are to be regarded as significant (odds 19 : 1). For the last three weeks of series IV, differences equal to about half the extremes shown in the graph satisfy this criterion. A test of the difference between the rates of sets A and D for the week of Dec. 22–29 gave odds of more than 99 : 1 for significance.

The manner in which the average holard for the different groups varied during the series is shown in the upper portion of figure 4. However, in this type of experiment it is possible that the roots might have been growing in the upper part of the soil mass only, reducing the holard of this region to a low value, with the result that the average holard obtained from calculations would not indicate the true moisture conditions under

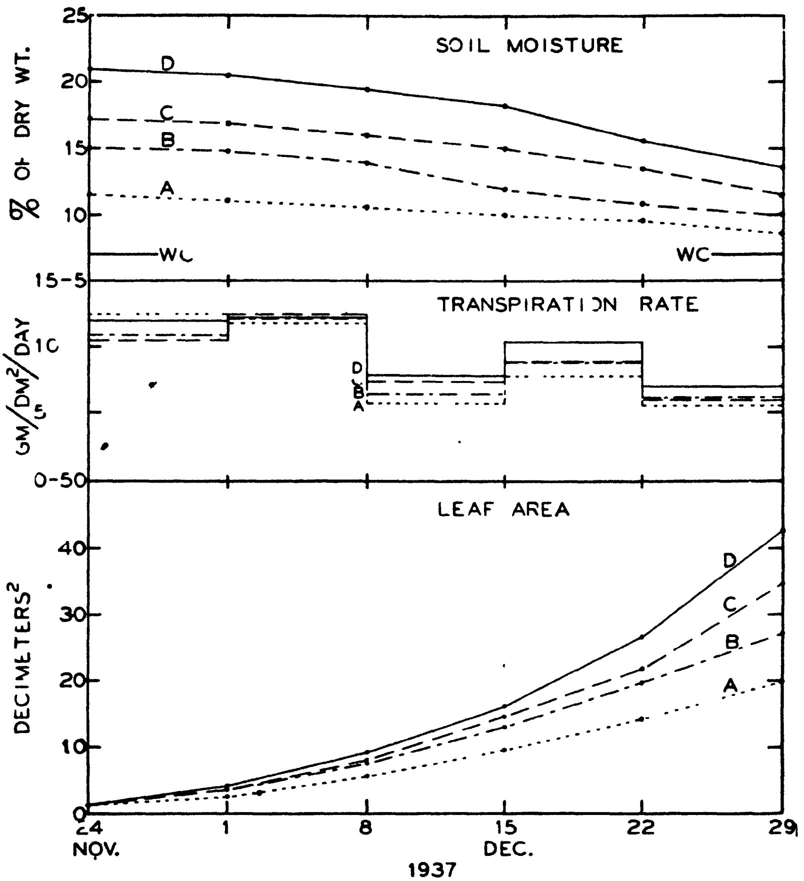


FIG. 4. Variation in soil moisture, transpiration rate, and leaf area during series IV. The values for the four groups with different initial holards are indicated by the letters A, B, C, and D.

which the plants were growing. In order to obtain some information concerning the distribution of moisture in the cans at various times during the series, samples were taken on Nov. 10, Dec. 22, and Dec. 29 at depths of 2, 8, 14, and 20 inches (bottom of the can), and the values obtained are recorded in table III. In all four sets, the tendency to maintain uniformity of holard at all depths is remarkable. Set D was initially given enough water to bring the average holard to greater than field capacity, with the result that there was an initial difference of 6.2 per cent. between the 2-inch and 20-inch levels. By Dec. 22, however, this difference had been reduced to 2.4 per cent. In the other three sets, the difference between top and bottom of the soil mass was less than 1 per cent. In none of the groups was there any evidence that the average holard of the upper parts

TABLE III

PERCENTAGES OF SOIL MOISTURE AT DIFFERENT DEPTHS IN THE CANS AT THE BEGINNING AND NEAR THE END OF SERIES IV. THE LETTERS A, B, C, AND D AT THE HEADS OF THE COLUMNS INDICATE THE GROUPS FOR WHICH THE INITIAL HOLARD WAS APPROXIMATELY 11, 14, 17, AND 20 PER CENT., RESPECTIVELY

DATE	DEPTH	A	B	C	D
	in.	%	%	%	%
	2	11.0	14.7	16.3	18.6
Nov. 10, 1937	8	11.8	15.1	17.0	19.8
	14	11.8	15.3	17.1	20.4
	20	11.9	15.6	18.3	24.8
	2	8.1	10.4	13.4	14.4
Dec. 22, 1937	8	8.7	11.1	13.6	14.8
	14	9.7	11.2	13.9	15.4
	20	9.2	10.5	13.2	16.8
	2	7.8	9.3	10.9	12.7
Dec. 29, 1937	8	9.8	10.2	11.5	12.9
	14	8.0	10.9	12.3	13.0
	20	8.7	9.1	10.9	15.1

of the soil mass had been reduced more than 1 or 2 per cent. before roots had penetrated to the bottom of the can.

In series V, soil samples were taken every week throughout the growing period, with results entirely in agreement with those of series IV. There appeared to be very little difference in rate of removal of water from different parts of the soil mass. Apparently the explanation of this phenomenon is that the roots were fairly well distributed throughout the soil mass by the time the plants had withdrawn enough water from the soil to reduce the average holard 1 or 2 per cent.

The variation of holard and leaf area during series V was similar to that in series IV, but the transpiration rates per unit leaf area behaved differently. Throughout the series the rate of transpiration of set A was from 10 to 15 per cent. greater than that of set D, with the other two sets intermediate, an order just opposite to that in series IV. This difference in behavior is presumably due to the fact that the plants in series V were much smaller at the end than were those in series IV, and accordingly had used much less water with less reduction in holard.

At the time the series were brought to a close, final measurements were taken, and the plants were cut down and dried in an electric oven at 65° C. The values obtained are given in table IV, and those from series IV are shown graphically in figure 2. In both series greater holards resulted in increased height, stem diameter, leaf area, dry weight of shoot and roots, percentage of water in the shoots, and total transpiration. There appeared to be no significant difference, however in shoot-root ratios. Water requirement behaved differently for the two series, increasing slightly with holard in series IV but decreasing in V.

TABLE IV

SUMMARY OF DATA OBTAINED FROM SERIES IV–V. ALL MEASUREMENTS ARE AVERAGES OF ALL PLANTS IN EACH GROUP. THE LETTERS A, B, C, AND D AT THE HEADS OF THE COLUMNS INDICATE THE GROUPS FOR WHICH THE INITIAL HOLARDS WERE APPROXIMATELY 11, 14, 17, AND 20 PER CENT., RESPECTIVELY

ITEM	SERIES IV				SERIES V			
	A	B	C	D	A	B	C	D
Number of plants in group	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Stem height (cm.)	32.0	40.0	41.0	51.0	16.0	18.5	26.8	29.2
Stem diameter (cm.)	1.09	1.24	1.44	1.63	0.64	0.78	1.04	1.20
Leaf area (dm.^{2})	19.7	27.0	34.7	42.7	5.92	9.70	19.5	25.0
Fresh weight of shoot (gm.)	74.0	120.0	154.0	201.0	18.9	33.2	77.2	93.2
Dry weight of shoot (gm.)	11.0	16.0	18.3	22.9	2.83	4.37	9.21	10.25
Dry weight of roots (gm.)	1.16	1.63	2.06	2.38	0.55	0.77	1.64	1.95
Dry weight, total (gm.)	12.2	17.6	20.4	25.3	3.38	5.14	10.85	12.20
Shoot/root, ratio	9.48	9.82	8.88	9.61	5.20	5.71	5.66	5.42
Percentage of water in shoot	85.1	86.7	88.1	88.6	85.1	86.8	88.1	89.0
Total transpiration (gm.)	2100.0	3110.0	3585.0	4675.0	785.0	1169.0	2408.0	2685.0
Water requirement (gm./gm.)	174.0	177.0	177.0	186.0	236.0	227.0	223.0	221.0
Number of stomata per mm.^{2}	339.0	308.0	262.0	185.0	394.0	325.0	285.0	228.0
Thickness of leaf (microns)	175.0	175.0	203.0	203.0	228.0	246.0	263.0	274.0
Palisade (percentage of total thickness)	52.0	52.0	52.0	52.0	57.0	57.0	53.0	55.0
Date of planting seeds	Oct. 30, 1937				Feb. 24, 1938			
Date of harvesting plants	Dec. 29, 1937				April 20, 1938			

Statistical tests show that for stem heights and diameters, total dry weights, and leaf areas, the odds are at least 19:1 that nearly all the differences between the means of adjacent groups are significant. For both series, however, the odds are only about 4:1 that the difference between the water requirements of sets A and D is real. It should be mentioned in this connection that, for both series IV and V, the order of variation of water requirement with holard was the same as that of rate of transpiration per unit area. In series IV, both water requirement and rate of transpiration increased with greater holard, while in series V the opposite order held. A similar relation was noted in series I–III.

The diurnal variation of stomatal opening was determined on Dec. 3, 13, and 28 for series IV. On the first date, no difference in behavior of the stomata of the various groups was apparent, but on the other two days, the stomata of set D remained a little more widely open in the afternoon than those of the other three sets. For series V, a determination of stomatal behavior on April 13 disclosed no difference in the various sets; all remained open all day. In general, the behavior of the stomata appeared to be closely related to the transpiration rate.

At the close of the series, stomatal counts made from strips of epidermis from the lower surface of the fourth pair of leaves showed that the number per square millimeter increased rapidly with decreasing holard, set A having nearly twice as many as set D. The size of the stomata, both in guard cells and in length of opening, was in inverse proportion to the frequency, those in set A being only little more than half as large as those in D. Sections through the mesophyll of the same leaves showed a reduction in size of the mesophyll cells with reduced holard. The leaves were also thinner in the latter situations but the proportion of the mesophyll composed of palisade was unchanged. The reduced anatomy of the leaves of plants growing in soil with lower holards appeared to be largely a result of failure to expand under lessened turgor, rather than to a difference in the number of initial cells formed.

Summary

1. Plants of the Russian Mammoth variety of *Helianthus annuus* have been grown in containers holding about 130 pounds of dry soil under conditions of different holard, using two methods.

(a) One group of plants was grown in soil in which the holard was maintained near the field capacity by frequent additions of water; the other group was allowed to remove about two-thirds of the available soil moisture before the supply was replenished.

(b) Four groups of plants were grown in soil mixed at predetermined holards; no water was added at any time during the series. The hollards selected were approximately 11, 14, 17, and 20 per cent. of the dry

weight of the soil. The wilting coefficient of this soil was 7 per cent. and the field capacity 18 per cent.

2. In all series lower holard resulted in reduced growth in terms of stem diameter, leaf area, dry weight, and usually in stem height.

3. In experiments using method (a) above, water requirement and shoot-root ratio increased with greater available water, but in those using method (b) neither was altered appreciably.

4. Stomata were smaller and more numerous on plants with less available water. The leaves were also thinner, but the proportions of the leaf composed of palisade and sponge tissue were unchanged. The leaf anatomy appeared to be largely a case of failure of the cells to expand because of the reduced turgor, rather than to a difference in the number of initial cells formed.

5. Growth rate was affected by even small differences in holard, according to both methods used, and was affected by reduced holard long before any effect on stomatal opening or transpiration rate per unit area could be detected.

6. The rate of transpiration per unit of leaf surface was ordinarily affected when about two-thirds of the available soil moisture had been removed, the actual point depending upon the transpiration rate. This was true even when large plants with closely spaced roots were used, and the transpiration rate was between 1 and 2 grams per square decimeter per hour.

7. The stomatal opening appeared to be unaffected unless the holard was reduced to the point where the transpiration rate was lowered.

8. In all cases in which the transpiration rate and stomatal opening were affected, the leaves showed signs of wilting.

9. Rate of increase in leaf area fell off as the soil moisture was reduced, but after watering recovered to a value greater than that of the control plants. This effect was probably due largely to recovery of turgor; if at all ascribable to this cause, however, then more than 18 hours were required for complete recovery.

10. No increase in the rate of transpiration per unit area of leaf surface of plants recovered from wilting above that of control plants, such as reported by TUMANOW, could be detected.

CARNEGIE INSTITUTION OF WASHINGTON
SANTA BARBARA, CALIFORNIA

LITERATURE CITED

1. BECKETT, S. H., BLANEY, H. F., and TAYLOR, C. A. Irrigation water requirement studies of citrus and avocado trees in San Diego County, California. California Agr. Exp. Sta. Bull. 489. 1930.
2. BRIGGS, L. J., and SHANTZ, H. L. The water requirement of plants.

II. A review of the literature. U. S. Dept. Agr. Bur. Plant Ind. Bull. 285. 1913.

3. CLEMENTS, F. E., and LONG, F. L. Factors in elongation and expansion under reduced light intensity. Plant Physiol. **9**: 767–781. 1934.
4. ———— and ————. Further studies of elongation and expansion in *Helianthus* by phytometers. Plant Physiol. **10**: 637–660. 1935.
5. HENDRICKSON, A. H., and VEIHMEYER, F. J. Irrigation experiments with peaches in California. California Agr. Exp. Sta. Bull. 479. 1929.
6. ———— and ————. Irrigation experiments with grapes. Proc. Amer. Soc. Hort. Sci. **28**: 151–157. 1931.
7. ———— and ————. Irrigation experiments with prunes. California Agr. Exp. Sta. Bull. 573. 1934.
8. ———— and ————. The irrigation of pears on a clay adobe soil. Proc. Amer. Soc. Hort. Sci. **34**: 224–226. 1937.
9. LEWIS, M. R., WORK, R. A., and ALDRICH, W. W. Studies of the irrigation of pear orchards on heavy clay soil near Medford, Oregon. U. S. Dept. Agr. Tech. Bull. 432. 1934.
10. ————, ————, and ————. Influence of different quantities of moisture in a heavy soil on rate of growth of pears. Plant Physiol. **10**: 309–323. 1935.
11. LLOYD, F. E. The physiology of stomata. Carnegie Inst. Washington Publ. no. 82. 1908.
12. MAGNESS, J. R. Status of orchard soil moisture research. Proc. Amer. Soc. Hort. Sci. **32**: 651–661. 1934.
13. ————, DEGMAN, E. S., and FURR, J. R. Soil moisture and irrigation investigations in eastern apple orchards. U. S. Dept. Agr. Tech. Bull. 491. 1935.
14. PESSIN, L. J. Effect of soil moisture on the rate of growth of longleaf and slash pine seedlings. Plant Physiol. **13**: 179–189. 1938.
15. SHANTZ, H. L. Drought resistance and soil moisture. Ecology **8**: 145–157. 1927.
16. TUMANOW, J. J. Ungenügende Wasserversorgung und das Welken der Pflanzen als Mittel zur Erhöhung ihrer Dürreresistenz. Planta **3**: 391–480. 1927.
17. VEIHMEYER, F. J. Some factors affecting the irrigation requirements of deciduous orchards. Hilgardia **2**: 125–291. 1927.
18. ————, and HENDRICKSON, A. H. Soil-moisture conditions in relation to plant growth. Plant Physiol. **2**: 71–82. 1927.
19. WORK, R. A., and LEWIS, M. R. The relation of soil moisture to pear tree wilting in a heavy clay soil. Jour. Amer. Soc. Agron. **28**: 124–134. 1936.

STORAGE OF ROOT RESERVES IN RHODES GRASS

H. WEINMANN

(WITH FIVE FIGURES)

Introduction

It is a well-known fact that the roots of many perennial herbaceous plants function as organs of storage, even where there are no special morphological modifications of the root system. Root-storage phenomena in herbaceous plants have been studied in detail by a number of workers. GRABER and associates (7) found that in lucerne, polysaccharides and nitrogen increased in percentage and amount in the roots during the mature stages whereas spring growth was associated with a decrease in the percentage of nitrogen and soluble sugars in both tops and roots. The authors conclude that "storage is greatly accelerated during the periods of blossoming and seed formation. At such periods, the plant produces reserve foods in excess of the amounts used in vegetative extension and a surplus is stored in the roots. . . ." WILLARD (20), likewise working on lucerne, made similar observations. ALDOUS (1) found that in the roots of *Vernonia baldwinii, Andropogon scoparius, Symphoricarpus vulgaris, Rhus glabra* and *Verbena stricta* the percentage of nitrogen, total sugars and starch in general decreased to about the time of flowering, after which considerable increases of these constituents were recorded. ALDOUS thinks that after the time of flowering "the plants begin to restore the reserves which must have been drawn on to produce the top growth." Similarly, GRANDFIELD (8) reports a 23.8 per cent. increase in the amount of total carbohydrates and a 16.8 per cent. increase in the amount of total nitrogen in the roots of *Verbena stricta* during the period between budding and maturity, and approximately the same increases in the roots of *Solidago rigida* and *Vernonia baldwinii.* According to BEWS and VANDERPLANK (5), the percentage of total carbohydrates increased during the entire season and that of total soluble sugars during winter only, in the corms of *Hypoxis rooperi,* while the percentages of the same compounds decreased in the leaves between spring and autumn. The authors think, however, that in this plant "the large carbohydrate reserves are drawn upon to only a very slight extent when new leaves are produced in spring."

More recently data have been collected with regard to the storage and utilization of root reserves in various grasses. STURKIE (16) found that the rootstocks of *Sorghum halepense* were developed after the maturing of aerial plant parts; this was confirmed by SAMPSON and McCARTY (14) in *Stipa pulchra,* where an accumulation of carbohydrate reserves in the roots was observed during maturation. In the roots of *Digitaria eriantha* and *Themeda*

triandra, BEWS and BAYER (4) found only somewhat irregular fluctuations of sugars and polysaccharides, whereas in *Elyonurus ambiguus* and *Muehlenbergia gracilis*, McCARTY (9) recorded an accumulation of sugars and starch in the stem bases, rhizomes and roots at the end of the season with the cessation of growth. That mineral elements may also be included in the substances stored in the roots, is shown by the work of RICHARDSON, TRUMBLE and SHAPTER (12, 13) on *Lolium subulatum* and *Phalaris tuberosa*. In both grasses, considerable losses of nitrogen, phosphorus, and potassium occurred from the herbage portions during maturity; amounting in *Phalaris tuberosa* to 36, 22, and 12 per cent. of the total intake of N, K_2O, and P_2O_5 respectively. In the case of *Phalaris tuberosa*, the losses of nitrogen and phosphorus could be accounted for by translocation to the basal internodes and to the root system; potassium was actually lost from all portions of the plant, amounting to 27.8 per cent. of the amount found in the entire plant shortly before flowering.

The writer, who has been engaged for several years in the study of the storage of root reserves in some South African Highveld grasses, found that in the roots of *Trachypogon plumosus, Tristachya hispida* and *Digitaria tricholaenoides*, the percentage of nitrogen and total sugars (17) as well as of phosphorus (unpublished data) decreased during the growing period and increased in autumn and winter. In connection with a study of the growth rhythm of the same three species and also of *Cynodon dactylon, Brachiaria serrata* and *Eragrostis chalcantha*, ALTONA (2) was recently able largely to confirm the results hitherto obtained by examining the percentages of nitrogen and total sugars in the roots of these grasses at their various stages of development. In general, the curves of nitrogen and total sugar content of the roots show a definite seasonal trend reaching a maximum in mid-winter and dropping at the time of shooting to reach a minimum at the time of flowering.

Seasonal chemical changes in the roots coincide largely with corresponding changes in the chemical composition of the herbage in the opposite direction. Decreases in the percentage of nitrogen, phosphorus, potassium and other constituents in the herbage during the growing season have been reported by many authors in various countries. When the total amounts of these constituents in the herbage per unit area are calculated, it is shown, however, that absorption continues for a considerable part of the growing season. While the decrease of the percentage of nitrogen, phosphorus and potassium during the greater part of the growing season is merely due to the progressive accumulation of carbohydrates, actual losses from the herbage of total amounts of these constituents occur during autumn when the percentages of the same constituents in the roots increase (17).

Naturally, mere percentage figures are not fully conclusive for the characterization of such processes as translocation and storage where various opposite processes or processes having opposite effects may go on at the same time. Thus, prolonged absorption of mineral elements by the roots from the soil may mask translocation of the same elements from shoots to roots, whereas an exceedingly high increase of one group of constituents may under certain circumstances result in a decrease of the percentage of another group, though that group may actually increase in amount. Only a determination of the total amounts of the various constituents present in shoots and roots at different stages of growth and maturation will indicate to what extent constituents have been translocated from shoot to root and vice versa. This, however, is rarely possible with plants growing in the field, since a collecting of the whole root bulk is mostly impracticable. For a more detailed investigation of the processes of absorption, translocation, and storage, pot experiments conducted under controlled conditions are more suitable.

The present contribution deals with the storage of root reserves in *Chloris gayana* KUNTH, Rhodes Grass, which was grown under controlled conditions in pots and was harvested at different stages of maturation.

Materials and methods

For this experiment cylindrical metal tins approximately 2 feet in height and with an open surface of 1250 cm^2 were used. These tins were first brought to equal weight by placing a few quartz stones in the bottom, and were then filled with ordinary veld soil collected at Frankenwald, the Botanical Research Station of the Witwatersrand University, near Johannesburg, South Africa, where the experiment was conducted.

The lower half of the tins was filled with 29.2 lb. of sub-soil consisting of coarse decomposed granite, the upper half with 32.2 lb. of top-soil (the figures expressing absolute dry weight). Top-soil as well as sub-soil were thoroughly mixed before being distributed into the single pots; the top-soil was also sieved through a wide-meshed sieve in order to exclude coarser stones. The top-soil used was an acid loamy sand, poor in organic matter and nutrients, particularly in nitrogen and phosphorus. The dry top-soil (as used) contained 29.85 per cent. stones (determined by using a sieve of 20 mesh-per-inch) and 2.35 per cent. organic matter (loss on ignition). The results of chemical and mechanical analyses of the top-soil fine earth are given in table I.

For the mechanical analysis the BOYOUCOS hydrometer method was used (6), nitrogen was determined by the ordinary Kjeldahl method, phosphorus and potassium were extracted by treating 100 gm. of air-dry soil with 200 ml. of 10 per cent. HCl on a boiling water bath for 3 hours. The maximum water retaining capacity of the top-soil (as used) was 34.0 per cent. and that

TABLE I

CHEMICAL AND MECHANICAL ANALYSES OF TOP-SOIL FINE EARTH. RESULTS GIVEN IN PERCENTAGE OF DRY MATTER

MECHANICAL ANALYSES			
PARTICLE SIZE IN MILLIMETERS			
SAND 1.0–0.05	SILT 0.05–0.005	CLAY < 0.005	TOTAL COLLOIDS < 0.008
% 76.5	% 8.1	% 15.4	% 16.0
CHEMICAL ANALYSES			
NITROGEN	PHOSPHORIC OXIDE	POTASH	
0.107	0.015	0.087	

of the sub-soil was 29.7 per cent., equalling approximately 20 lb. of water per pot.

Thirty six tins were filled with soil as described above; one series of 12 tins received no fertilizer treatment (0); another series of 12 tins received a fertilizer treatment in the form of sulphate of ammonia, phosphate, and potash salt (PNK); and the third series of 12 tins were given the same treatment as the second with the difference that a threefold quantity of ammonium sulphate was given (PN_3K). The actual amounts of nutrients applied per pot were as follows:

$P = 6$ gm. raw rock and superphosphate mixture (27 per cent.) $= 1.44$ gm. P_2O_5;

$K = 1.2$ gm. muriate of potash (60 per cent.) $= 0.72$ gm. K_2O;

$N = 3$ gm. sulphate of ammonia (21.1 per cent.) $= 0.63$ gm. N;

$N_3 = 9$ gm. " " " " $= 1.89$ gm. N.

The phosphate was distributed and mixed into the soil surface about one inch deep on Dec. 15, 1936; sulphate of ammonia and potassium chloride were applied in the form of a solution on Dec. 17; on the 18th a number of seeds of Rhodes Grass were sown into each pot. Germination was satisfactory and after about one month the seedlings were singled out so that finally ten plants remained in each pot.

In contrast to the plants of the PNK and PN_3K series the plants of the 0 series were very poor from the beginning, and it was feared that they would die on account of a marked deficiency of nutrients. In order to prevent this,

to ten pots of this series a small amount of PNK equalling 1/20 of the proper PNK treatment was given on January 25, 1937; two pots of this series were kept entirely unfertilized as an absolute control. The whole experiment thus consisted altogether of four treatments:

	0	pnk	PNK	PN_3K
Number of pots	2	10	12	12

It later became evident, however, that the plants of the 0 series overcame their first set back and did not die; on the other hand, the small pnk treatment was not able to produce any significant difference as compared with the 0 plants. Practically, both of these series can be regarded as control series; the plants of both series remained true "deficiency" plants.

The plants were kept in the open air but were, in the early stages of growth, sheltered against heavy storms by means of a movable roof of corrugated iron which was removed as soon as rain had ceased. For watering, collected rain water was used and the water content of the pots was kept more or less constant by frequent weighing. The water supply was controlled according to the requirements of the growing plants. Thus, when the seeds were sown into the pots the soil possessed a water content equalling 33 per cent. of the maximum water retaining capacity of the pots; the water content was raised to 50 per cent. of the M.W.R.C. on Dec. 30, to 60 per cent. on Feb. 13, to 70 per cent. on March 2, and to 75 per cent. on April 3. On April 17, when the plants approached maturity, all watering was stopped and the pots were allowed to dry out gradually.

The plants were harvested at three different stages of maturity; namely, three to four pots of each series on March 23, April 17, and May 18, 1937. The two pots of the absolute control series (0) were harvested on the last date as well. The total herbage of each pot was first cut off directly at the base including also the subaerial parts of the stems, and the whole root system was collected by washing it free from the surrounding soil. Care was taken to avoid losses of roots, and at the same time to shorten the procedure of washing as much as possible, in order to avoid any risk of loss of constituents from the tissue by leaching. Roots and shoots were then air dried in a glass house. When air-dry, their weight was determined; hereafter the material of one treatment and harvest—roots and shoots separately—was united and finely ground. The ground material was stored in airtight bottles and kept for the various determinations.

The following constituents were determined in shoots and roots: Total sugars, total hydrolyzable carbohydrates, nitrogen, phosphorus, potassium and soluble ash.

Total sugars.—5 gm. of the airdry substance were (after the addition of 1 gm. $CaCO_3$) extracted twice with 200 ml. of 95 per cent. alcohol for a

period of two hours under a reflux condenser. The residue was then removed by filtering and the alcohol distilled off under reduced pressure; the remaining syrup was taken up with distilled water, cleared with neutral lead acetate, and deleaded with potassium oxalate. The extract was then hydrolyzed by boiling with HCl under a reflux condenser for 1¼ hours (2.5 ml. conc. HCl were added to 100 ml. solution). Hereafter the solution was neutralized and made up to 500 ml. volume. The reducing power of this extract was then estimated by the MUNSON and WALKER method (**10**) followed by the BERTRAND method for the estimation of cuprous oxide (**3**). From the amount of copper precipitate the glucose equivalent was found by reference to the tables of MUNSON and WALKER.

Total hydrolyzable carbohydrates.—As recommended by BEWS and VANDERPLANK (**5**), 5 gm. of airdry substance were hydrolyzed with 200 ml. of 1.1 per cent. HCl in a beaker, covered with a watch glass, on a boiling water bath for 4½ hours. The hydrolyzed and filtered extract was then neutralized, cleared and deleaded, made up to 500 ml. volume, and its reducing power determined as described above. The results were also expressed as glucose.

Nitrogen.—Nitrogen was estimated by the ordinary Kjeldahl method, using a mixture of K_2SO_4 and $CuSO_4$ (9 : 1 parts by weight) as a catalyst in the digestion.

Phosphorus.—A sample of the plant material was incinerated after the addition of saturated lime water in order to avoid losses of organic phosphorus in the ignition. After removal of the silica, phosphates were precipitated in the nitric acid extract of the plant ash as ammonium phosphomolybdate according to LORENZ (**18**).

Potassium.—Potassium was determined as potassium perchlorate in the HCl extract of the plant ash after removal of silica, sulphates, phosphates, calcium, iron, aluminium, and magnesium (**18**).

Soluble ash.—A weighed ash sample of the plant material was treated with hot HCl. The liquid was then filtered off and the remaining insoluble residue (SiO_2) was washed, ignited, and weighed. The difference total plant ash minus SiO_2 was reported as soluble ash.

Dry matter.—The dry matter content was determined by drying a separate sample in an electric oven for 12 hours at a temperature of 105° C. All results of the chemical determinations were expressed as percentages of dry matter.

Results

YIELDS AND GENERAL DEVELOPMENT OF THE PLANTS

In their general development and growth rhythm, the plants of the PNK and PN_3K series were soon far in advance of the plants of the 0 and pnk series. The leaves of the PN_3K plants had a darker green color than the

leaves of the other plants, owing to their high nitrogen supply; the plants of 0 and pnk series, on the other hand, were poor and yellowish, and were not able to form stems and inflorescences. Both, PNK and PN_3K plants, produced stems and inflorescences, but the PN_3K plants did not only form more bulk than the PNK plants but produced also a larger number of inflorescences per plant (see fig. 1). The final average number of inflorescences formed by

FIG. 1. Inflorescences produced under varying nutrients. From left to right:
pnk series: received 1/20 of proper PNK treatment;
PNK series: received proper PNK treatment;
PN_3K series: three fold quantity of ammonium sulphate.
(Photographed April 17, 1937).

[illegible]n plants (as counted on April 17 after the flowering period was finished) was 10.5 and 27.2 for PNK and PN_3K plants respectively. Maturation was accelerated by both these fertilizer treatments: the plants of PNK and PN_3K treatment possessed relatively early (middle of March) a fairly large proportion of dead, yellowish leaves—PN_3K plants again more than PNK plants—whereas 0 and pnk plants remained in the green, vegetative stage throughout the growing season, though yellowing occurred here to a certain extent, probably owing to deficiency of nutrients.

Three to four pots of each series were harvested at three different stages of maturation, namely on March 23, April 17, and May 18, 1937, respectively. At the first date, flowering was just commencing, while at the second date the flowering period was definitely finished. The first frosts were recorded on April 22, 24, and 29, followed by frosts on every day during May with the exception of a warm spell from the 6th to the 14th. The third harvesting date (May 18), was, therefore, well after the growing season had

ceased. At this last date, the PN_3K plants possessed only very few green leaves, the PNK plants a few more, whereas the plants of 0 and pnk series were still almost entirely green. While it is frequently observed that nitrogen in excess retards maturation, the earlier maturation of the PN_3K plants in this case may be partly due to the greater daily fluctuations in the water content of the pots of these better developed plants owing to their higher total transpiration. Seeding was in full swing near the last harvesting date and many seeds had fallen before this day. Further losses of seeds in handling the plants were unavoidable to a certain extent but, as far as possible, seeds were collected and included in the samples.

The results of the yield determinations—expressed as grams dry weight of shoots, roots, and total plant matter per pot (ten plants)—are given in table II and represented in figure 2. The figures are averages of all three or

TABLE II

YIELD OF PLANTS GROWN UNDER VARIOUS NUTRIENT CONDITIONS. GRAMS OF DRY MATTER PER TEN PLANTS

DATE	0	pnk	PNK	PN_3K
		SHOOTS		
	gm.	*gm.*	*gm.*	*gm.*
March 23, 1937	....	8.6 ± 0.5	78.7 ± 2.9	121.1 ± 1.3
April 17, 1937	....	14.0 ± 0.7	83.8 ± 8.2	136.3 ± 6.3
May 18, 1937	15.5 ± 1.4	16.3 ± 0.7	87.3 ± 3.9	130.0 ± 3.5
		ROOTS		
March 23, 1937	....	13.9 ± 1.4	51.4 ± 2.5	71.3 ± 3.2
May 17, 1937	....	25.8 ± 4.2	62.6 ± 2.6	74.3 ± 3.0
May 18, 1937	19.9 ± 0.6	21.2 ± 1.0	77.3 ± 4.0	92.4 ± 4.8
		TOTAL		
March 23, 1937	....	22.5 ± 1.5	130.1 ± 3.8	192.4 ± 3.5
April 17, 1937	....	39.8 ± 4.3	146.4 ± 8.6	210.6 ± 7.0
May 18, 1937	35.4 ± 1.5	37.5 ± 1.2	164.6 ± 5.6	222.4 ± 6.0

four pots harvested at each date and are given together with their corresponding standard errors. The standard error (s) was calculated by means of the formula:

$$s = \pm \sqrt{\frac{n(n-1)}{\Sigma v^2}},$$

where Σv^2 = sums of the squares of the deviations of the single observations from the mean, and n = number of single observations.

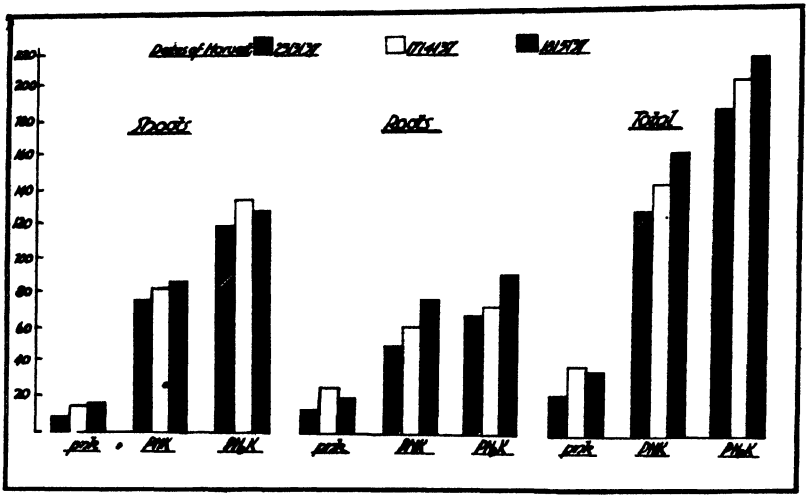


FIG. 2. Yields; grams of dry matter per ten plants.

A comparison of the yields of the three sampling dates shows that with regard to the shoots, growth was practically finished on April 17, no significant difference being between the yields of April 17, 1937 and May 18, 1937 with the possible exception of the pnk plants which show also during this period a slight and perhaps significant increase of 2.3 gm. ± 0.99. The growth rhythm was, however, different in the *roots*—at least in PNK and PN_3K plants—which also increased in the very last part of the growing season, *i.e.*, after flowering and during formation of seeds. The changes in the root system between April 17 and May 18 were as follows:

in pnk a decrease of 4.6 gm. ± 4.3 (= − 17.8 per cent.);
in PNK an increase of 14.7 gm. ± 4.8 (= + 23.5 per cent.);
in PN_3K an increase of 18.1 gm. ± 5.7 (= + 24.4 per cent.).

The decrease is small and uncertain for its relatively high error in the pnk plants. At any rate, the pnk plants were, on account of a general deficiency of nutrients, hindered in their normal development. But significant increases of the root system occurred in the well-fertilized plants during the late stages of maturation, when aerial growth had already ceased. This is in agreement with observations of several other workers already mentioned.

CHEMICAL CHANGES IN THE PLANTS DURING MATURATION

The results of the chemical determinations are given in table III and figure 3. The figures show that in the shoots the percentage of most constituents determined decreased during maturation. This is particularly distinct with regard to sugars, hydrolyzable carbohydrates, phosphorus and potassium[1];

[1] Potassium and soluble ash could not be determined in the pnk series owing to lack of sufficient material.

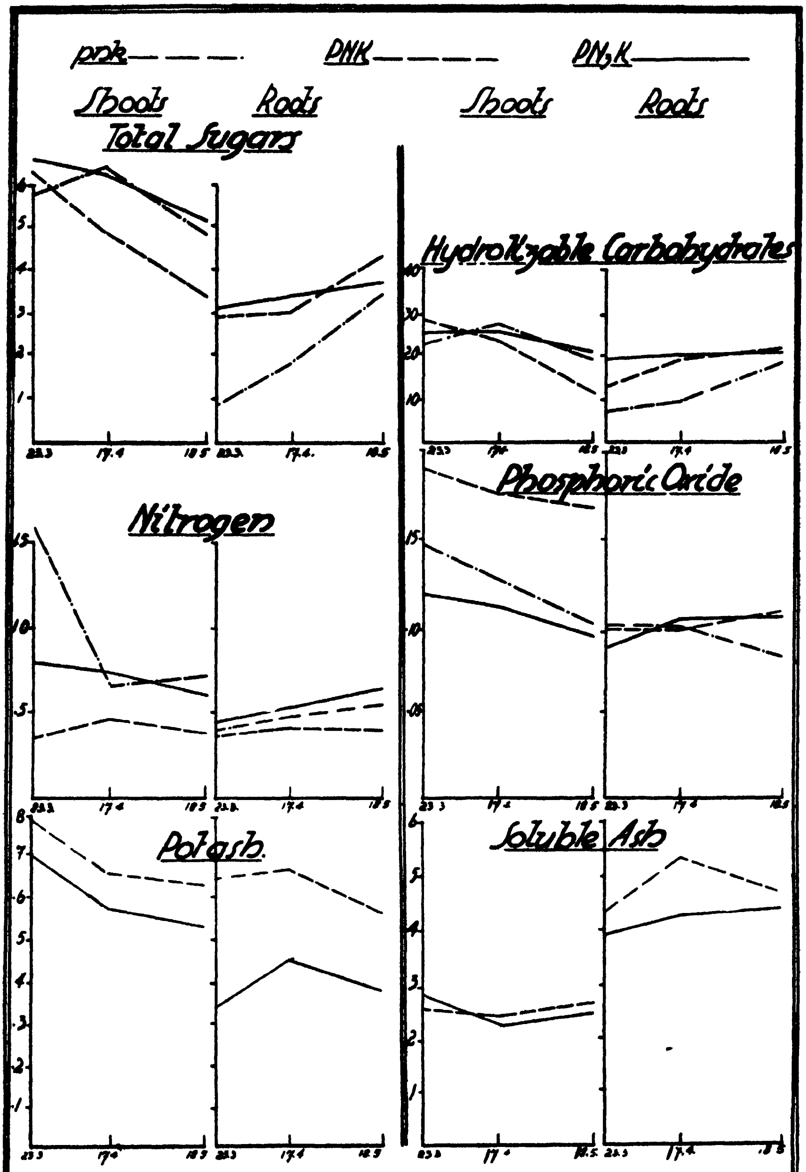


FIG. 3. Chemical composition of shoots and roots; percentages of dry matter.

percentage nitrogen decreased distinctly at least in the PN_3K series, while the percentage of soluble ash[1] changed only little. In the roots, however, the percentages of most constituents determined increased during maturation,

TABLE III

CHEMICAL COMPOSITION OF SHOOTS AND ROOTS.
EXPRESSED AS PERCENTAGE OF DRY MATTER

CONSTITUENT	DATE	pnk		PNK		PN_3K	
		SHOOTS	ROOTS	SHOOTS	ROOTS	SHOOTS	ROOTS
		%	%	%	%	%	%
Total Sugars	3/23/37	5.80	0.97	6.52	2.90	6.55	3.04
	4/17/37	6.34	1.87	4.72	2.97	6.25	3.31
	5/18/37	4.77	3.38	3.32	4.37	5.06	3.73
Hydrolyz. Carbohy-drates	3/23/37	23.25	8.06	29.50	14.00	25.50	20.65
	4/17/37	27.20	10.17	23.75	20.27	26.50	21.00
	5/18/37	20.75	19.20	11.95	21.90	21.25	21.75
Nitrogen	3/23/37	1.55	0.41	0.35	0.36	0.81	0.47
	4/17/37	0.65	0.48	0.48	0.40	0.72	0.52
	5/18/37	0.73	0.53	0.37	0.39	0.59	0.62
Phosphoric Oxide	3/23/37	0.149	0.104	0.192	0.096	0.122	0.087
	4/17/37	0.127	0.097	0.178	0.096	0.113	0.099
	5/18/37	0.097	0.083	0.167	0.108	0.093	0.105
Potash	3/23/37	......	......	0.77	0.63	0.69	0.33
	4/17/37	......	......	0.65	0.64	0.56	0.44
	5/18/37	......	......	0.62	0.55	0.52	0.36
Soluble Ash	3/23/37	......	......	2.52	4.28	2.74	3.90
	4/17/37	......	......	2.38	5.27	2.20	4.19
	5/18/37	......	......	2.53	4.69	2.45	4.35

though in some cases the changes were considerably smaller than in the shoots. Thus, in the roots of the PN_3K plants the percentages of sugars and hydrolyzable carbohydrates increased only slightly. The same holds true for nitrogen in all series and for phosphorus in the PNK and PN_3K series, while the percentage of potassium decreased in the roots of both well fertilized series towards the end of maturation and that of soluble ash in the PNK series only.

From the percentage figures and the dry weight of shoots and roots, the actual amounts of constituents in shoots and roots of each treatment and date were calculated. The results are given in table IV, expressed as grams of constituents per ten plants and represented in figure 4. As indicated above, the calculation of total amounts offers a much better means of deciding whether a constituent has actually decreased or increased in a portion of a plant, since a change in the percentage of a constituent does not necessarily mean that a corresponding change of the actual amount of the constituent in the plant or plant part has occurred.

With the exception of soluble ash, which remained more or less constant over the whole period of maturation, all constituents decreased distinctly in

TABLE IV

ACTUAL AMOUNTS OF CONSTITUENTS IN SHOOTS AND ROOTS. EXPRESSED AS GRAMS PER TEN PLANTS

CONSTITUENT	DATE	pnk			PNK			PN_3K		
		SHOOTS	ROOTS	TOTAL	SHOOTS	ROOTS	TOTAL	SHOOTS	ROOTS	TOTAL
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Total sugars	3/23/37	0.50	0.13	0.63	5.13	1.49	6.62	7.95	2.17	10.12
	4/17/37	0.89	0.48	1.37	3.96	1.86	5.82	8.53	2.46	10.99
	5/18/37	0.78	0.71	1.49	2.88	3.38	6.26	6.57	3.45	10.02
Hydrolyz. carbohydrates	3/23/37	2.0	1.1	3.1	23.2	7.2	30.4	30.9	14.7	45.6
	4/17/37	3.8	2.6	6.4	19.9	12.7	32.6	36.2	15.6	51.8
	5/18/37	3.4	4.1	7.5	10.4	16.9	27.3	27.6	20.1	47.7
Nitrogen	3/23/37	0.13	0.06	0.19	0.28	0.16	0.44	0.98	0.33	1.31
	4/17/37	0.09	0.12	0.21	0.40	0.25	0.65	0.98	0.39	1.37
	5/18/37	0.12	0.11	0.23	0.32	0.30	0.62	0.77	0.57	1.34
Phosphoric oxide	3/23/37	0.013	0.014	0.027	0.151	0.049	0.200	0.148	0.062	0.210
	4/17/37	0.018	0.025	0.043	0.149	0.060	0.209	0.154	0.074	0.228
	5/18/37	0.016	0.018	0.034	0.146	0.084	0.230	0.121	0.097	0.218
Potash	3/23/37	......	......	......	0.61	0.32	0.93	0.84	0.24	1.08
	4/17/37	......	......	......	0.54	0.40	0.94	0.76	0.33	1.09
	5/18/37	......	......	......	0.54	0.43	0.97	0.68	0.33	1.01
Soluble ash	3/23/37	......	......	......	1.98	2.20	4.18	3.32	2.78	6.10
	4/17/37	......	......	......	1.99	3.30	5.29	3.00	3.12	6.12
	5/18/37	......	......	......	2.21	3.63	5.84	3.18	4.02	7.20

actual amount in the shoots of the well fertilized and normally developed plants of the PNK and PN_3K series, particularly during the last part of maturation. In the roots, on the other hand, all constituents increased in total amount—in most cases right through the period of maturation. Some exceptions are found in the pnk plants which may be explained by their abnormal development owing to a general deficiency; as mentioned above, these plants never reached the stage of physiological maturation.

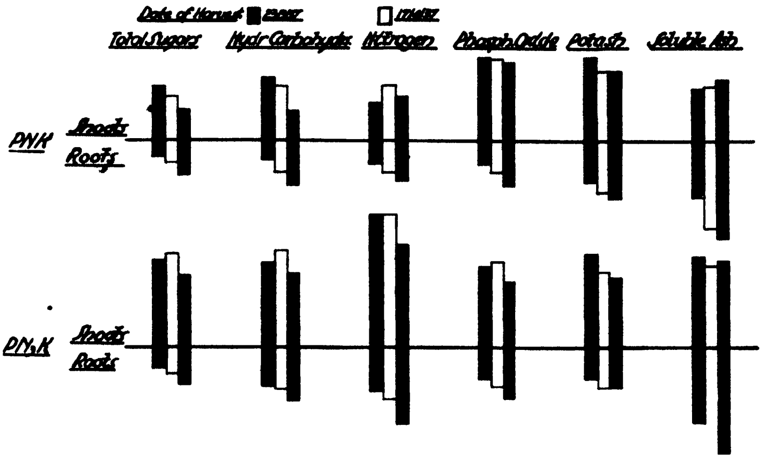


FIG. 4. Actual amounts of constituents (different scales).

If the total amounts of nitrogen and mineral elements present in the entire plant at different stages of maturation are compared with each other, it is revealed that absorption must have continued at least for a part of the maturation period. The differences in physiological maturation between PNK and PN_3K plants are well reflected in their total uptake of nitrogen and mineral elements. Nitrogen absorption was practically completed by March 23 in the PN_3K series, whereas in the less advanced PNK plants considerable absorption of nitrogen took place until April 17; in the immature remaining pnk plants nitrogen absorption continued until May 18. Similarly, absorption of phosphorus continued in the PNK series up to the last sampling date on May 18, but had already finished in the PN_3K series on April 17. Potassium increased very slightly during maturation in the PNK plants, but no absorption of potassium occurred during the same period in the more advanced PN_3K plants. In the latter series, there was even a loss of total amount of potassium during the last part of maturation, amounting to 0.08 gm. K_2O per ten plants. Though this small loss cannot be regarded as significant, since the variations of the total dry matter between pots of the same harvest would account for an error of $\pm$ 0.045, it should be remembered again

that substantial losses of potash from all portions of the plant during the late stages of maturation have been recorded by RICHARDSON, TRUMBLE and SHAPTER (13) in *Phalaris tuberosa.* Similar losses of nitrogen and mineral elements by remigration into the soil during maturation are also reported by WILFARTH, RÖMER and WIMMER (19), SEKERA (15) and PENSTON (11).

The figures of soluble ash are only of limited physiological value, since some mineral elements (such as phosphorus and sulphur) are partly lost in the ignition and are therefore not quantitatively included. It would appear, however, from the total amounts of soluble ash that absorption continued right through the period of maturation, again more pronounced in the PNK series than in the PN_3K series. In both series, the bulk of mineral elements absorbed during this period accumulated only in the roots, not appreciably affecting percentages and amounts of soluble ash in the shoots.

It should be remembered that in the interpretation of the data presented a number of actual and possible errors must be taken into account. The standard error of the dry matter (as given in table II) would naturally appear proportionally as standard error of each single constituent of the dry matter, limiting the conclusions as to the significance of the differences recorded. Possible variations in the chemical composition of plants of the same treatment and harvest but from different pots are not even included here, since only combined samples were analyzed. In addition, some inherent errors in methods would probably influence the results at least slightly: small amounts of plant matter were lost at the end of maturation by the loss of seeds and rain may have leached out constituents from dead leaves.

Furthermore, as far as carbohydrates are concerned, one would not expect that the constituents disappearing from the shoots should be recovered in exactly the same amounts in the roots. Sugars translocated from shoots to roots may there be polymerized to various types of polysaccharides, part of which (such as pentosans, hemi-celluloses and celluloses) do not appear as "hydrolyzable carbohydrates" under the conditions of hydrolysis employed. On the other hand, photosynthetic activity followed by immediate translocation of the photosynthetic products to the roots, would result in an increase of the amounts of carbohydrates in the roots without a corresponding decrease in the shoots. Appreciable losses of carbohydrates may possibly occur in the respiration of living plant parts, particularly also of actively growing roots, or in the decomposition of dead parts.

While for all these reasons too far-reaching conclusions should be avoided, the results should be conclusive proof that in *Chloris gayana* during the period of maturation appreciable amounts of organic reserves as well as of mineral elements are translocated from shoots to roots, in part accompanied by continued contemporaneous absorption by the roots of nitrogen and mineral nutrients from the soil.

The continuous accumulation of dry matter and constituents effected in this way, in the root portion of the plants after flowering, becomes most evident when the proportions of root dry matter and of root constituents are

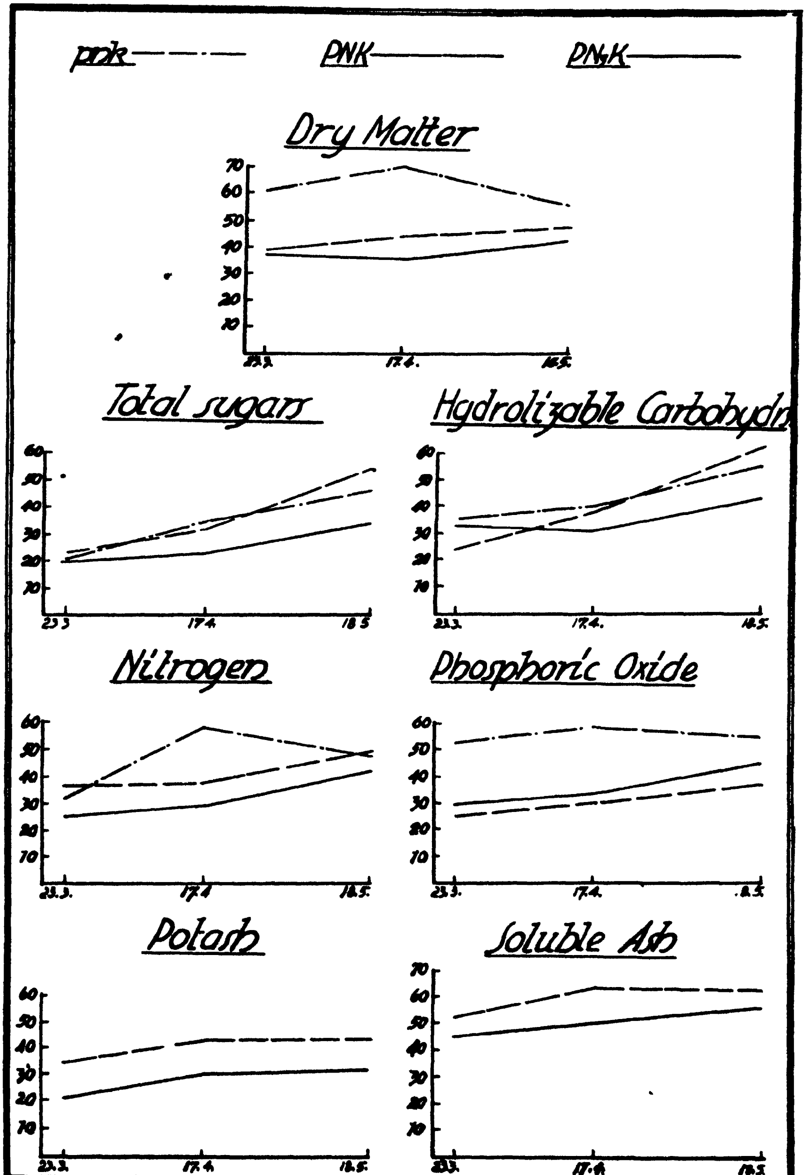


FIG. 5. Relative amounts of dry matter and constituents in the roots; percentages of the total amount in the plant.

expressed as percentages of the corresponding amounts present in the entire plant $\left(\frac{\text{amount in roots} \times 100}{\text{amount in whole plant}}\right)$. As can be seen from table V and figure 5,

TABLE V

RELATIVE AMOUNTS OF DRY MATTER AND CONSTITUENTS IN THE ROOTS. EXPRESSED AS PERCENTAGES OF THE TOTAL AMOUNT IN THE PLANT

CONSTITUENT	DATE	pnk	PNK	PN_3K
		%	%	%
Dry matter	3/23/37	61.8	39.6	37.1
	4/17/37	64.8	42.8	35.3
	5/18/37	56.6	47.0	41.5
Total sugars	3/23/37	20.7	22.5	19.8
	4/17/37	35.0	32.0	22.4
	5/18/37	47.7	54.0	34.4
Hydrolyz. carbohydrates	3/23/37	35.5	23.7	32.2
	4/17/37	40.7	38.9	30.1
	5/18/37	54.7	61.9	42.2
Nitrogen	3/23/37	31.6	36.4	25.2
	4/17/37	57.2	38.5	28.5
	5/18/37	47.8	48.4	42.5
Phosphoric oxide	3/23/37	51.9	24.5	29.5
	4/17/37	58.2	28.7	32.5
	5/18/37	52.9	36.5	44.5
Potash	3/23/37		34.4	22.2
	4/17/37		42.6	30.3
	5/18/37		44.3	32.7
Soluble ash	3/23/37		52.6	45.6
	4/17/37		62.4	51.0
	5/18/37		62.2	55.8

these relative amounts of dry matter and constituents in the roots increased distinctly during maturation, the only exceptions occurring in the pnk series, probably owing to the retarded development of these deficiency plants.

Summary

Chloris gayana KUNTH, Rhodes Grass, was grown under controlled conditions in pots receiving different fertilizer treatments and was harvested at three different stages of maturity.

In the well fertilized plants significant increases of the root system occurred after the time of flowering and during the formation of seeds, when growth of the aerial parts had already ceased.

During this period considerable amounts of sugars, hydrolyzable carbohydrates, nitrogen, phosphorus and potassium were lost from the shoots and were largely recovered in the roots.

In addition to translocation from the shoots, nitrogen and mineral elements also accumulated in the roots to a certain extent by simultaneous absorption from the soil. The duration of the absorption period varied, however, for different elements; it also varied for plants grown under different nutritional conditions and hence reaching different stages of physiological maturation.

The relative amounts of dry matter and constituents present in the roots (expressed as percentages of the total amounts present in the whole plant) increased, in most cases, considerably during autumn, indicating a continuous storage of these substances in the roots with the approach of maturity.

Abnormally developed and immature remaining deficiency plants showed in part a correspondingly abnormal behavior with regard to root storage and absorption.

The writer is greatly indebted to PROF. J. PHILLIPS, Head of the Department of Botany of the University of the Witwatersrand, for his interest and the facilities provided in his Department; to PROF. H. STEPHEN, Head of the Department of Chemistry, for placing a laboratory and apparatus at his disposal; to DR. B. SEGAL of the same Department for frequent advice and to MR. A. H. BUNTING for his assistance in the analytical work. Sincere thanks are also accorded to the National Research Council and Board of South Africa for having assisted the writer with a grant in connection with this research.

UNIVERSITY OF THE WITWATERSRAND
JOHANNESBURG, SOUTH AFRICA

LITERATURE CITED

1. ALDOUS, A. E. Relation of organic food reserves to the growth of some Kansas pasture plants. Jour. Amer. Soc. Agron. **22**: 385–392. 1930.
2. ALTONA, R. E. A report of an investigation of the seasonal movements of root reserves in six highveld grasses. Unpublished B.Sc.Hons. Thesis, Univ. of the Witwatersrand. 1939.
3. BERTRAND, G. Le dosage des sucres réducteurs. Bull. Soc. Chim., ser. 3, **35**: 1285–1299. 1906.
4. BEWS, J. W., and BAYER, A. W. Researches on the vegetation of Natal—Series III, Section I. On the reaction of *Digitaria eriantha* var. *stolonifera* (Stapf) and *Themeda triandra* (Forsk) to the winter season. South African Jour. Sci. **28**: 158–168. 1931.
5. ———, and VANDERPLANK, J. E. Storage and other carbohydrates in a Natal succulent and a Natal geophyte and their behavior before, during, and after the winter resting season. Ann. Bot. **44**: 689–719. 1930.

6. BOUYOUCOS, G. J. A comparison of the hydrometer method and the pipette method for making mechanical analysis of soils, with new directions. Jour. Amer. Soc. Agron. **22**: 747–751. 1930.
7. GRABER, L. F., NELSON, N. T., LEUKEL, W. A., and ALBERT, W. B. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisconsin Agr. Exp. Sta. Res. Bull. 80. 1927.
8. GRANDFIELD, C. O. The relation of organic food reserves to the effect of cutting pasture weeds at different stages of growth. Jour. Amer. Soc. Agron. **22**: 709–718. 1930.
9. MCCARTY, E. C. Seasonal march of carbohydrates in *Elyonurus ambiguus* and *Muehlenbergia gracilis* and their reaction under moderate grazing use. Plant Physiol. **10**: 727–738. 1935.
10. MUNSON, L. S., and WALKER, P. H. The unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663–686. 1906.
11. PENSTON, N. L. Return of mineral elements to the soil by plants. Nature **136**: 268–269. 1935.
12. RICHARDSON, A. E. V., TRUMBLE, H. C., and SHAPTER, R. F. Factors affecting mineral content of pastures. Council Sci. & Ind. Res., Australia, Bull. 49. 1931.
13. ———, ———, and ———. The influence of growth stage and frequency of cutting on the yield and composition of a perennial grass—*Phalaris tuberosa*. Council Sci. & Ind. Res., Australia, Bull. 66. 1932.
14. SAMPSON, A. W., and MCCARTY, E. C. The carbohydrate metabolism of *Stipa pulchra*. Hilgardia **5**: 61–100. 1930.
15. SEKERA. Der Mineralstoffwechsel der Gerste. Zeitschr. Pflanzenernähr., Düngung & Bodenk. B, **7**: 535. 1928.
16. STURKIE, D. G. The influence of various top-cutting treatments on root stocks of Johnson grass (*Sorghum halepense*). Jour. Amer. Soc. Agron. **22**: 82–93. 1930.
17. WEINMANN, H. Investigations on the storage of root reserves in some South African grasses. Unpublished M.Sc. Thesis, Univ. of the Witwatersrand. 1938.
18. WIESSMANN, H. Agrikulturchemisches Praktikum. Paul Parey, Berlin. 1926.
19. WILFARTH, R., RÖMER, H., and WIMMER, G. Über die Nährstoffaufnahme der Pflanzen in verschiedenen Zeiten ihres Wachstums. Landw. Vers.-Sta. **63**: 1–70. 1906.
20. WILLARD, C. J. Root reserves of alfalfa with special reference to time of cutting and yield. Jour. Amer. Soc. Agron. **22**: 595–602. 1930.

THE MINERAL COMPOSITION OF THE ALCOHOLIC EXTRACT OF POTATO LEAVES AND ITS RELATIONSHIP TO CROP YIELDS

C. E. BEAUCHAMP

(WITH THREE FIGURES)

Introduction

In previous papers on the nutrition of the sugar-cane plant (3, 4), it was shown that the mineral composition of the alcoholic extracts of cane leaves taken at an early stage of growth, from plots having different fertilizer treatments, bore a direct relationship to growth and final yield. Growth was measured in terms of average stalk length, average number of stalks per stool, and total cane weight. In that investigation it was shown that the composition of the skeleton of the leaves deprived of the minerals soluble in alcohol, had no correlation at all with cane growth. These results were checked in another fertilizer experiment (2) conducted in an adjacent field consisting of the same soil type and using the same cane variety. The interesting results obtained in these investigations prompted the writer to study the mineral composition of alcoholic extracts of potato leaves taken from a potato fertilizer experiment.

The experiment was started on February 8, 1936, on a Matanzas red clay. This soil type is very deep and friable, the difference between soil and sub-soil being scarcely noticeable. Its pH was about 6.5. The potato variety planted was Irish Cobbler. Plots of 1/47 acre were 99 by 9.30 feet. Each plot comprised five rows 1.86 feet apart.

The fertilizer was applied at planting time at the rate of 1500 lb. per acre. Eight different treatments were compared including checks, each one being replicated three times at random.

The fertilizer treatments compared were as follows:

No. 1—Check	No. 5— 8–10–15
No. 2—6– 8–10	No. 6—10– 8– 0
No. 3—8–10–10	No. 7—10– 8–10
No. 4—8–10– 0	No. 8—10– 8–15

The fertilizer mixtures were prepared as shown in table I.

On March 30, 1936, fifty-two days after planting, leaf samples were taken from each plot. Leaves from similar treatments were mixed. Aliquots, enough to fill a one-quart, wide-mouthed bottle, were used. 95 per cent. alcohol was poured in so as to cover the leaf material. All bottles were then pasteurized at 60° C. for 20 min. and sealed. The green matter of the leaves was removed using Soxhlet extractors. The extraction was

TABLE I

FERTILIZER MIXTURES USED IN GROWTH OF IRISH COBBLER POTATOES

FORMULA	POUNDS PER ACRE	PLANT FOOD			UREA	NITRATE OF SODA	SULPHATE OF AMMONIA	DOUBLE SUPER-PHOSPHATE	SULPHATE OF POTASH	MURIATE OF POTASH
		NH_3	P_2O_5	K_2O						
	lb.	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*	*lb.*
6–8–10	1500	90	120	150	27	160	180	267	156	123
8–10–10	1500	120	150	150	27	160	300	333	156	123
8–10–0	1500	120	150	......	27	160	300	333	....	.
8–10–15	1500	120	150	225	27	160	300	333	234	184
10–8–0	1500	150	120	....	27	160	420	267	....	....
10–8–10	1500	150	120	150	27	160	420	267	156	123
10–8–15	1500	150	120	225	27	160	420	267	234	184

discontinued when the alcoholic leachings came out colorless. The quantity of leaf material taken from each treatment was arbitrary. The amount of alcohol used for each sample was also variable depending upon the volume necessary to effect a complete extraction. The alcoholic extracts were then transferred to beakers and evaporated on the water bath. The residues from the alcoholic extracts as well as the leaf skeletons were dried to constant weight. The former were digested with sulphuric acid until colorless. The volume was then made up to 500 ml. and kept in sealed bottles until analyzed.

Methods

NITROGEN

Twenty-five ml. aliquots of the solution were digested for 2 hours with 5 ml. of H_2SO_4 plus 2 gm. of K_2SO_4 and 0.02 gm. $CuSO_4$ in a 250-ml. Kjeldahl flask.

The samples were transferred to the VanSlyke-Cullen aeration apparatus, and about 18 ml. of saturated NaOH were added. Aeration was continued for four hours into 200 ml. of standard H_2SO_4 (0.01990 N). Back titration was made with NaOH of the same normality.

PHOSPHORUS

The procedure as outlined in the methods of the Association of Official Agricultural Chemists (1935) was used on 50-ml. aliquots of the solution. Titration was made with 0.05140 N NaOH and 0.02570 N HNO_3.

POTASSIUM

The 25-ml. aliquots were evaporated to dryness and heated to remove H_2SO_4. Dehydration, to remove SiO_2, was made by adding 4–5 ml. of con-

centrated HCl, evaporating to dryness on the steam bath, heating for one hour and taking up the residue in hot H_2O. The dehydrated SiO_2 was removed by filtration, and calcium was precipitated as the oxalate. The solutions were again filtered, evaporated to dryness, heated to expel ammonium salts, and K_2O determined by the chloroplatinic method as described in the A.O.A.C., 1935.

Calcium

62.5-ml. aliquots were evaporated and heated to remove H_2SO_4. The residues were taken up in H_2O, and calcium was precipitated as described in A.O.A.C., 1935, by adding 5 ml. of saturated $(NH_4)_2C_2O_4$.

The CaC_2O_4 was removed by filtration through an asbestos prepared Gooch. After dissolving the precipitate in H_2SO_4 (1:2), titration was made with $KMnO_4$. This was very weak and was made up twice, with normalities of 0.011517 and 0.01161.

Magnesium

Magnesium was determined in the filtrate from calcium by precipitation with 8-hydroxyquinoline. The solutions were made to about 150 ml., adjusted to neutrality, and heated to 70–80° C. Four ml. of 5 per cent. 8-hydroxyquinoline in 2 N acetic acid were added. Two ml. in excess of 1:2 NH_4OH were added, and the solutions were allowed to stand for one hour until the precipitate had settled. Filtration was made through sintered glass crucibles, and the precipitate was washed well with cold H_2O.

The crucibles containing the precipitate were dried to constant weight at 105° C. The percentage of magnesium was calculated from the weight of $Mg\ (C_9H_6NO)_2 \cdot 2H_2O$ obtained.

Harvesting

On April 17, 1936, the potatoes were dug. The yield from each plot was carefully weighed and classified into no. 1's and no. 2's according to size.

Results

Table III shows the yield response of the potatoes to the different fertilizer treatments applied. The yield of the check plots was insignificant. All fertilizer mixtures resulted in very high increases when compared with check plots. Among the fertilized plots, the differences in yield were not marked, yet they were sufficient to show how the crop responded to the various fertilizer treatments.

The increments of potash in the fertilizers were followed by a marked yield increase as shown by comparing yields of treatments 8–10–0, 8–10–10, 8–10–15 and 10–8–0, 10–8–10, and 10–8–15. In the first three treatments

TABLE II

YIELD OF "NUMBER 1" AND "NUMBER 2" POTATOES GROWN IN EXPERIMENTAL PLOTS

Treatments		Plot number	Yield per plot No. 1	Yield per plot No. 2	Total	No. 1	No. 2
			lb.	lb.	lb.	%	%
1.	Check	5	8	38	46	17.0	83.0
		17	4	24	28	14.0	86.0
	Average		6	31	37	15.5	84.5
2.	6–8–10	3	280	22	242	90.9	9.1
		7	190	16	206	92.2	7.8
	Average		205	19	224	91.6	8.4
3.	8–10–10	9	209	18	227	92.0	8.0
		11	190	24	214	88.8	11.2
	Average		199.5	21	220.5	90.4	9.6
4.	8–10–0	1	177	28	205	86.3	13.7
		8	114	18	132	86.3	13.7
		12	129	16	145	98.0	11.0
	Average		140	21	161	87.2	12.8
5.	8–10–15	2	246	21	267	92.0	8.0
		6	235	21	256	92.0	8 0
		18	182	15	197	92.4	7.6
	Average		221	19	240	92.1	7.9
6.	10–8–0	13	153	16	169	90.5	9.5
		15	164	16	180	91.1	8.9
		19	141	17	158	89.2	10.8
	Average		152.7	16.3	169	90.3	9.7
7.	10–8–10	10	221	11	232	95.3	4.7
		14	205	14	219	93.7	6.3
	Average		213	12.5	225.5	94.5	5.5
8.	10–8–15	4	274	20	294	93.3	6.7
		16	224	16	240	93.3	6.7
		20	222	15	237	93.7	6.3
	Average		240.0	17.0	257.0	93.5	6.5

the yields were 79.43, 105.98 and 120.79 cwt. per acre respectively. In table II, it is seen that the percentage of no. 1 potatoes increased slightly but consistently with increments of potash in the fertilizers.

Increments of nitrogen in this experiment did not increase yields. With 10 per cent. of potash and 8 per cent. of phosphoric acid an increase of nitrogen from 6 to 10 per cent. NH_3, did not have any effect at all on the

TABLE III

AVERAGE YIELDS PER ACRE

TREATMENTS		WEIGHTS PER ACRE		
		No. 1	No. 2	TOTAL
		cwt.	*cwt.*	*cwt.*
1.	Check	2.82	14.57	17.39
2.	6–8–10	96.35	8.93	105.28
3.	8–10–10	93.76	9.87	103.63
4.	8–10–0	65.80	9.87	75.67
5.	8–10–15	103.87	8.93	112.80
6.	10–8–0	71.77	7.66	79.43
7.	10–8–10	100.11	5.87	105.98
8.	10–8–15	112.80	7.99	120.79

yields. This is brought out in table III by comparing yields of plots treated with mixtures 6–8–10 and 10–8–10.

In comparing treatments with 8 and 10 per cent. of ammonia it is unfortunate that the content of phosphoric acid varies; 10 per cent. in the first case and 8 per cent. in the second. In spite of this irregularity there is practically no significant difference between the yields of treatments 8–10–0 and 10–8–0, being 75.67 and 79.43 cwt. per acre, respectively. The same holds in comparing treatments 8–10–10 and 10–8–10; the respective yields being 103.63 and 105.98 cwt. per acre. There appears to be a slight increase in yield as a result of a nitrogen increment in treatment 10–8–15 as compared with 8–10–15. In these cases the yields were 120.79 and 112.80 cwt. per acre, respectively. This difference, however, did not prove to be significant when subjected to statistical analysis.

No comparison can be made between phosphorus increments in this experiment because both phosphorus and nitrogen vary at the same time. It was shown above, however, that increasing the nitrogen did not result in yield increases. In the case of phosphorus, table III shows that an increase of this nutrient had a negative effect on the yield. This is brought out by comparing yields from treatments 10–8–10 *vs.* 8–10–10 and 10–8–15 *vs.* 8–10–15. From these comparisons it cannot be definitely decided whether phosphorus increments depressed yields as a result of a low nitrogen content in the fertilizer or if the nitrogen increments were ineffective due to a lower phosphorus content.

The analyses of the alcoholic extracts of the leaves of the various treatments appear in table IV.

The figures in this table are very significant. If the yields per acre are compared with the total percentage of nutrients in the dry matter of the leaf extracts a very good correlation is observed. This correlation is readily seen in figure 1. The lowest yield obtained in the check plots and in

TABLE IV

Composition of the leaf extracts in percentage of dry extract. Average of two determinations

Treatments	Yield per acre	K_2O	P_2O_5	N	Ca	Mg	Total
	cwt.	%	%	%	%	%	%
1. Check	17.39	2.87	0.155	0.429	0.079	0.115	3.648
2. 6–8–10	105.28	9.77	0.460	0.974	0.095	0.337	11.638
3. 8–10–10	103.63	10.76	0.534	1.042	0.084	0.432	12.852
4. 8–10–0	75.67	3.97	0.233	1.034	0.126	0.427	5.790
5. 8–10–15	112.80	11.29	0.509	1.027	0.084	0.321	13.231
6. 10–8–0	79.43	5.84	0.237	1.306	0.106	0.654	8.143
7. 10–8–10	105.98	11.27	0.515	1.050	0.095	0.507	13.437
8. 10–8–15	120.79	9.97	0.568	1.208	0.079	0.390	12.215

turn, the leaf extracts from these plots, showed the lowest percentage of total nutrients, *i.e.*, nitrogen, phosphorus, potash, calcium, and magnesium.

Next to the check plots in low yields stand those receiving fertilizers without potash, nos. 4 and 6. In these cases both the yields and the percentage of nutrients in the alcoholic extracts of the leaves were higher than in the check plots, but considerably lower than in plots receiving complete fertilizers.

Plots receiving complete fertilizer mixtures (nos. 2, 3, 5, 7, and 8) showed the highest potato yields and also the highest content of total nutrients in the alcoholic extracts of the leaves.

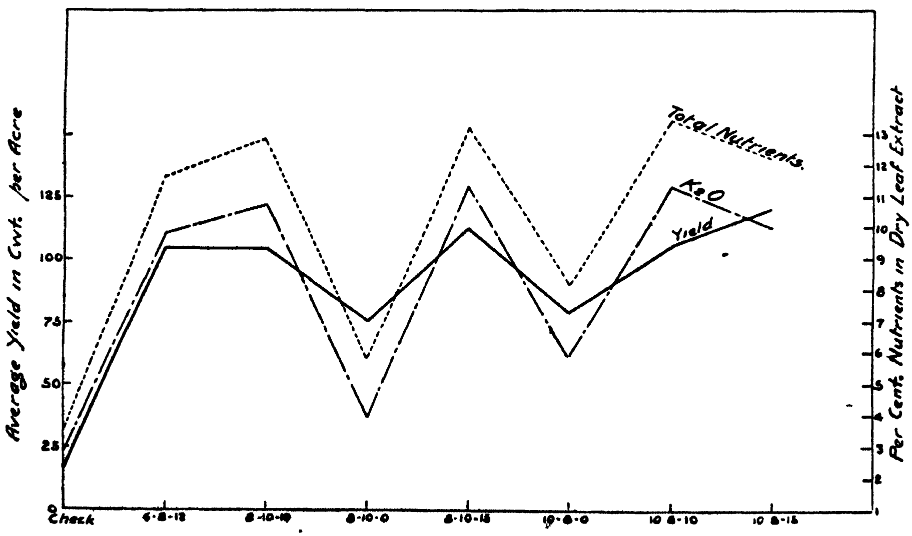


Fig. 1. Yield of potatoes in relation to potassium supply.

If the contents of the individual elements comprising the total nutrients are correlated with yields obtained, it will be seen that table IV presents very interesting information. The content of potash in the alcoholic leaf extracts was directly correlated with the amount of this element applied in the fertilizer and again with the yields obtained (fig. 1). The lowest content of potash resulted in the leaf extract of the check plots. The highest content of this nutrient was found in the leaf extracts of plots treated with complete fertilizers. Therefore, the application of potash in the fertilizer resulted in an increase of this nutrient in the alcoholic leaf extract and also in an increased yield. Treatments with no-potash fertilizers present an interesting case. Even though the amount of potash absorbed by the plant and actually found in the alcoholic leaf extract was considerably lower than in the case of plots receiving fertilizers with potash, it was higher than in the check plots. The crop yields followed the same sequence. They were lower than those resulting from the complete fertilizers but very much higher than the check plots. The crop received nitrogen and phosphorus, and these elements were absorbed by the plants and found in the leaf extracts in a higher proportion than in the check plots. It appears, therefore, that the mere absorption of more nitrogen and phosphorus stimulated the plants to absorb more potash. If this nutrient had been present in the soil in adequate amounts, the crop would have absorbed a larger amount to meet its requirements, or to balance the other elements.

It is also interesting to observe among the fertilized plots that the nitrogen absorption was not handicapped by withholding potash from the fertilizers. On the contrary, this element was evidently absorbed by the plant uninterruptedly and thus it is present in the alcoholic extract of the leaves, irrespective of the amount of potash present in the fertilizer.

Phosphorus, on the other hand, acts differently. Its absorption is apparently dependent on, or limited by, the amount of potash absorbed by the plant. This is shown in table IV by comparing columns representing the contents of both phosphorus and potash in the alcoholic extracts of potato leaves. For convenience these figures are plotted in figure 2. This graph shows plainly that as the content of potash in the alcoholic extract of the leaves increases, the amount of phosphorus in the extract also increases. When potash was withheld from the fertilizer, however, the contents of both potash and phosphorus in the alcoholic leaf extract remained low even though the phosphorus content of the fertilizer was maintained at a high level. These facts would indicate that phosphorus cannot be efficiently used by the potato crop if the potash supply of the soil is deficient.

The contents of both calcium and magnesium in the alcoholic extract of potato leaves bear an inverse relation to that of potash (fig. 3). The percentage of each of these elements tends to decrease as that of potash increases and *vice-versa.*

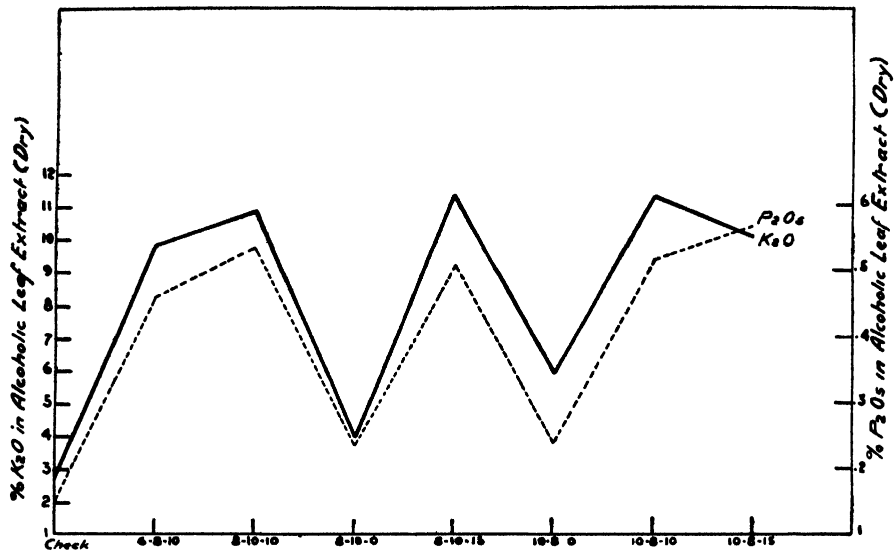


FIG. 2. Relationship between phosphorus and potassium in alcoholic extracts of potato leaves.

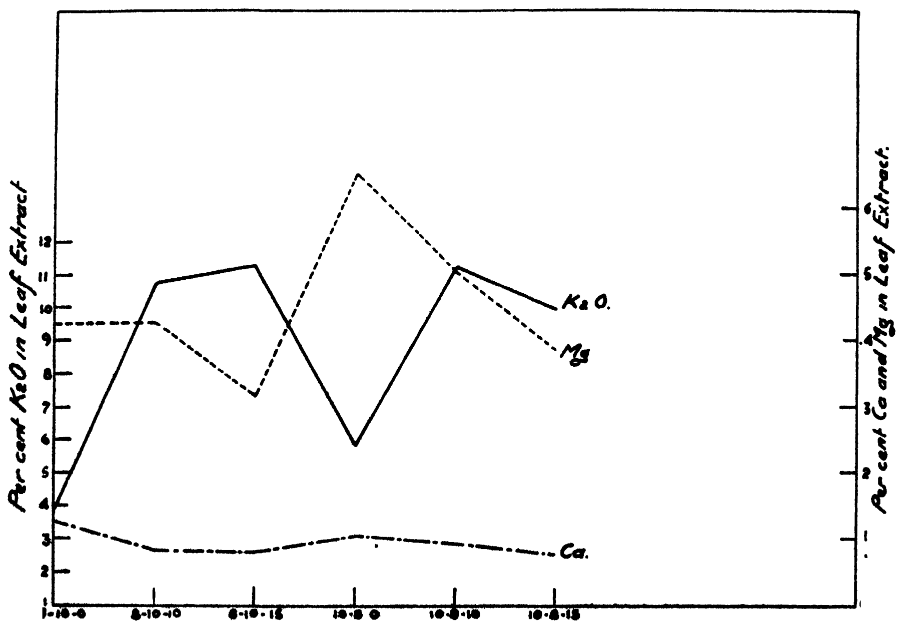


FIG. 3. Relationship between calcium, magnesium, and potassium in alcoholic extract of potato leaves.

Quantitative and qualitative relationships of the minerals in the alcoholic leaf extracts to crop yields

Table IV and figure 1 show that the total yield per acre is directly correlated with the total amount of minerals in the alcoholic extract of the leaves. The lowest yields invariably corresponded with the lowest percentages of total nutrients in the alcoholic extract of the leaves, and *vice-versa,* the highest yields obtained in treatments showed the highest percentage of total nutrients. These facts indicate that the percentage of total nutrients absorbed by the plant and concentrated in the leaves at the right time determines the productiveness of that plant at harvest time. Consequently, a quantitative relationship is involved between the amount of nutrients in the alcoholic leaf extract and crop yields.

The percentage of the different nutrients in the mineral portion of the leaf extract varies according to the different treatments. Table V shows the percentage composition of the mineral portion.

TABLE V

Percentage of nutrients in the mineral portion of the leaf extract

Treatments	Yield per acre	K_2O	P_2O_5	N	Ca	Mg	Total
	cwt.	%	%	%	%	%	%
1. Check	17.39	78.67	4.25	11.76	2.17	3.15	100.00
2. 6–8–10	105.28	83.96	3.95	8.37	0.82	2.90	100.00
3. 8–10–10	103.63	83.72	4.16	8.11	0.65	3.36	100.00
4. 8–10–0	75.67	68.57	4.02	17.86	2.18	7.37	100.00
5. 8–10–15	112.80	85.33	3.84	7.78	0.63	2.42	100.00
6. 10–8–0	79.43	71.72	2.91	16.04	1.30	8.03	100.00
7. 10–8–10	105.98	83.87	3.83	7.82	0.71	3.77	100.00
8. 10–8–15	120.79	81.62	4.65	9.89	0.65	3.19	100.00

From this table it is seen that the percentage of potash in the mineral portion of the leaf extract is lower in the check plots and in those receiving no-potash fertilizers. In the latter it is still lower than in the former. The percentage of potash was highest in plots receiving complete mixtures. In this case, there is little variation between treatments with 10 and 15 per cent. potash in the fertilizers.

The phosphorus content of the mineral portion is variable and is not consistent in relation to the different treatments. The lowest figure is found in plots treated with a no-potash mixture. It is highest in plots fertilized with the 10–8–15 formula. Among the remaining treatments it is more or less the same, but its content in the check plots is higher than in the latter.

The percentage of nitrogen in the mineral portion of the leaf extract is noteworthy. It appears to be highest in the check plots and in those

fertilized with the no-potash mixtures. It is considerably lower in plots receiving the complete fertilizer mixtures. The fact that the lowest yielding plots had a higher nitrogen content in the mineral portion of the leaf extract indicates that under a deficiency of potash this element is not completely assimilated and thus converted into plant tissues or fruits. Consequently, a state of equilibrium or balance of the mineral elements in the leaf is essential for an efficient metabolism.

The content of calcium in the mineral portion of the leaf extracts is much higher in the check plots and in no-potash plots than in those to which complete fertilizers were applied. In this respect calcium resembles nitrogen.

The amount of magnesium in the mineral portion of the leaf extracts also resembles that of calcium and nitrogen in that it is higher in plots receiving fertilizers without potash than in plots fertilized with the complete mixtures. In the check plots, however, the ratio of magnesium in the mineral portion of the leaf extracts did not increase to a relatively high figure as in the case of no-potash plots. It is rather within the limits of the figures found in the leaf extracts from plots receiving complete fertilizer mixtures.

Table V shows that the contents of both calcium and magnesium in the mineral portion of the alcoholic extracts of potato leaves vary inversely with the contents of potash in the leaves and in the fertilizer. When no potash was applied, the content of this nutrient in the mineral portion of the leaf extracts was relatively low and, in turn, the percentages of calcium and magnesium in the latter were relatively high. On the other hand, as the percentage of potash in the mineral portion of the leaf extract increased, the contents of calcium and magnesium decreased.

These facts give weight to the opinion of the writer, and other investigators, that an equilibrium between the minerals within the plant is essential for optimum growth and productivity. Moreover, this mineral equilibrium or plant food balance, shows both quantitative and qualitative relationships.

Lagatu and Maume (**11, 12, 13, 14**), Thomas (**22, 23**) and others (**19**) use the expression "intensity of nutrition" to represent the sum of the percentages of nitrogen (N), potash (K_2O) and phosphoric acid (P_2O_5) in dried foliage. This factor represents the quantity of nutrition at the moment of leaf sampling. Likewise they represent the quality of nutrition by the so-called "N–P–K unit." This unit is obtained by expressing in milligram equivalents the percentages of the three elements on the dried foliage, determining the proportion in which these elements are found in the total of their respective values and multiplying by 100 to avoid fractions. These two concepts, intensity of nutrition and N–P–K units, have been found to be intimately related with differential fertilizer treatments and their corresponding yields.

In order to confirm the importance and usefulness pertaining to the researches of LAGATU and MAUME, THOMAS, and their collaborators, their concepts of intensity of nutrition and N–P–K units are applied in this work to the mineral composition of the dry alcoholic extract of potato leaves although it is admitted that the above mentioned investigators base both magnitudes on the composition of the dried leaf.

In table VI are summarized the yields per acre, the intensity of nutrition and the N–P–K units as found in the dry alcohol leaf extract.

TABLE VI

YIELDS PER ACRE, INTENSITY OF NUTRITION, AND N–P–K UNITS IN DRY ALCOHOL LEAF EXTRACT

TREATMENTS	YIELD PER ACRE	INTENSITY OF NUTRITION	N–P–K UNITS		
		$N + P_2O_5 + K_2O$	N	P_2O_5	K_2O
	cwt.	%			
1. Check	17.39	3.454	31.15	6.67	62.18
2. 6–8–10	105.28	11.204	23.41	6.55	70.04
3. 8–10–10	103.63	12.336	22.81	6.92	70.27
4. 8–10–0	75.67	5.237	43.88	5.86	50.26
5. 8–10–15	112.80	12.826	21.87	6.42	71.71
6. 10–8–0	79.43	7.383	40.96	4.40	54.64
7. 10–8–10	105.98	12.835	22.26	6.47	71.27
8. 10–8–15	120.79	11.746	27.09	7.55	65.36

This table shows that there is a very high correlation between the yields of the different plots and the corresponding values expressing the intensity of nutrition and the N–P–K units as determined from the composition of the dry alcohol leaf extract. Taking the check treatment as a reference point, the above figures show that any fertilizer treatment resulting in an increased yield raised the intensity of nutrition and modified the N–P–K unit. Fertilizer treatments resulting in more or less similar yields show similar values for their intensity of nutrition and N–P–K units. Moreover, these figures indicate that both quantity and quality of nutrition are involved in the yield of the potato. Check treatments as compared with complete fertilizer treatments show a very low intensity of nutrition and an N–P–K unit having a higher proportion of nitrogen and a lower ratio of potash. No-potash treatments compared with check treatments show a higher intensity of nutrition but a highly unbalanced N–P–K unit, the K_2O content of which is lower but with a higher proportion of nitrogen. On the other hand, the addition of potash to the fertilizer resulted in a higher intensity of nutrition and a better balanced N–P–K unit, the latter having a higher K_2O content and a lower nitrogen content.

The facts derived from this investigation point to the following generalizations: An optimum yield in the potato is characterized by an optimum intensity of nutrition and an optimum equilibrium in the N–P–K unit as determined from the mineral composition of the dry alcohol leaf extract. Yields are lower than the optimum when the intensity of nutrition is low or when the N–P–K unit is unbalanced or when both of these conditions obtain in respect to the corresponding magnitudes for the optimum yielding plots.

Discussion

Several investigators (**11, 12, 13, 14, 20, 23, 24**) have found a correlation between the mineral composition of plant leaves, crop yields, and fertilizer applications. The works of LAGATU and MAUME (**11, 12, 13, 14**), MAUME and DULAC (**19**), THOMAS (**22, 23**), and others are outstanding in this respect.

In 1934, BEAUCHAMP, LAZO, and BONAZZI (**3, 4**) correlated the mineral composition of similarly located leaves from sugar cane plots, receiving differential fertilizer treatments, with crop yields. Both the alcohol-soluble and alcohol-insoluble leaf portions were analyzed separately. It was found that there was a definite correlation between the mineral content of the alcohol-soluble leaf portion and cane growth with its subsequent yield and the fertilizer treatments effected. On the other hand, the composition of the leaf skeleton or the alcohol-insoluble leaf portion did not show any such correlations.

In another investigation conducted by BEAUCHAMP and LAZO (**2**) on sugar cane, the mineral composition of the alcoholic extract of the leaves was found to be directly correlated with cane growth and yield. Direct correlations were found between the total mineral content found and cane growth as measured by average stalk length, average number of stalks per stool, average stalk weight, total cane weight and the percentage of available sugar in the juice. Increments of potash in the fertilizer resulted in increasing amounts of this element in the alcohol-soluble leaf portion and in turn, in increasing cane growth in terms of the above mentioned indices. On the other hand, increments of phosphorus or nitrogen in the fertilizer did not result in increasing amounts of these nutrients in the alcoholic leaf extracts or in a better response of the cane in the field.

This same method of alcoholic extraction was followed in the present study with the potato crop. It is well to consider that the succulent green potato leaves liberate considerable water to the alcoholic medium; the extracting liquid thus consists of a water-alcohol mixture. Other experiments are in progress to determine the effect of different ratios of water and alcohol on the efficiency of the mineral extraction. Further studies are also being conducted to determine other methods of extraction since

the Soxhlet process is time-consuming, especially when a large leaf sample is used.

The facts reported in this investigation show that the composition of the alcoholic extract of potato leaves is intimately correlated with crop yields and with the different fertilizer treatments applied. Analysis of the data obtained also confirms the results secured by LAGATU and MAUME (**11, 12, 13, 14**), MAUME and DULAC (**19**), THOMAS (**22, 23**) and others, working with entire leaves. These investigators found a definite correlation between the mineral composition of crop leaves, yields, and fertilizer treatments. Furthermore, the study here reported brings to light the antagonism, or reciprocal relationships, existing between various nutrients.

The yield of potatoes per acre varied directly with the total percentage of nitrogen plus phosphorus, potash, calcium and magnesium in the alcoholic extracts of the leaves. A low mineral content in the alcoholic extract corresponded with a low yield of potatoes per acre; a high mineral content corresponded with a high yield. This fact is important from a practical standpoint since it reflects the fertility level of the soil and thus the productive capacity of the crop grown in that soil. A low fertility level resulted in a low mineral content in the alcoholic leaf extract and *vice-versa*. Any significant yield increase resulting from a fertilizer application was correlated with a significant increase in the mineral content of the alcoholic leaf extract. Conversely, the mineral content of the latter did not change significantly when the fertilizer applied failed to produce a significant yield increase. Increments of potash in the fertilizer resulted in increasing percentages of this nutrient in the alcoholic extract and, in turn, in the yield of potatoes per acre. Neither nitrogen nor phosphorus increments in the fertilizer increased the amounts of these nutrients in the alcoholic leaf extracts, but they increased the content of potash in the latter as compared to the potash content of the leaf extracts of the check plots. This resulted in a higher nutrient content and hence in better yields than the check plots.

The percentage of phosphorus, however, in the alcohol-soluble leaf portion increased concomitantly with increases of potash in same, irrespective of the phosphorus content of the fertilizer. When no potash was applied, the content of both potash and phosphorus in the alcoholic extracts dropped to a very low level, despite the fact that the phosphorus content of the fertilizer remained unchanged. The absorption of nitrogen by the alcohol-soluble leaf portion was not affected by withholding potash from the fertilizer. These facts confirm the results obtained by BEAUCHAMP, LAZO, and BONAZZI (**3, 4**), and by BEAUCHAMP and LAZO (**2**), in the sugar cane investigations.

In the present study it is seen that increments of potash decreased the contents of both calcium and magnesium in the alcohol leaf extracts. This

agrees with the findings of numerous investigators on the question of antagonism (**1, 5, 6, 7, 9, 10, 15, 16, 17, 18, 20, 21, 25**). In an important research, THOMAS and MACK (**24**), found that there was a definite relationship between the contents of K_2O, CaO, and MgO within the leaves of corn and potato plants. When the content of potash increased, calcium and magnesium decreased, and *vice-versa.* These investigators arrived at the conclusion that when either of these elements is not present in sufficient amounts in the soil, the plant responds by a relatively excess absorption of one of the others, resulting in a disequilibrium which is reflected in reduced yields.

In the mineral portion of the alcohol leaf extracts, it is seen that nitrogen, calcium, and magnesium increase as the content of K_2O decreases. When no potash was applied the percentage of these elements in the mineral portion of the alcoholic extracts increased to very high levels but the resulting yields were low. On the other hand, when yields were high the content of potash in the mineral portion of the alcohol leaf extract was high while that of nitrogen, calcium, and magnesium was low. These facts indicate that an optimum percentage of potash in the alcohol-soluble leaf portion is essential for an efficient utilization of those three nutrients in plant metabolism.

The present investigation affords eloquent evidence as to the quantitative and qualitative relationships between soil fertility and crop yields as has been reported by numerous investigators. When the supply of plant nutrients in the soil was low the mineral content of the alcohol-soluble leaf portion was low and low yields resulted even if the ratio between the various nutrients departed little from normal. On the other hand, yields were also low when the plant food supply of the soil was unbalanced. In that case the ratio between the different nutrients in the mineral portion of the alcohol soluble leaf portion was abnormal.

The magnitudes representing the intensity of nutrition and the N–P–K units used by LAGATU, MAUME, and THOMAS quoted above as derived from the composition of the dried foliage were applied in the present instance to the composition of the dry alcoholic leaf extract and the values so obtained confirm the results of those investigators to the effect that both the quantity and quality of nutrition are involved in an optimum yield. Consequently, the analysis of the values so obtained afford an adequate means of correlating fertilizer treatments or soil fertility with crop yields.

The results of this investigation make it evident that the study of the mineral composition of the leaves, or different leaf portions, of cultivated crops may become a very useful tool in the hands of the agronomist or the plant physiologist to correlate soil fertility with crop yields thus formulating a sound basis for judicious fertilizer recommendations.

Summary

1. Increments of potash in the fertilizer resulted in marked increases in yield.

2. Increments of nitrogen and phosphorus did not produce any increase in yield.

3. The field yields of potatoes showed a very good correlation with the total percentage of plant food nutrients found in the alcoholic extracts of the leaves. Leaf samples were taken 52 days after planting, and harvesting was effected 16 days after sampling. A low total percentage of nutrients in the dry matter of the alcohol leaf extract always resulted in low yields, and conversely, a high percentage of total nutrients in same resulted in high yields.

4. The increase of potash in the fertilizer resulted in an increase in the percentage of this element in the alcohol leaf extract and, in turn, a higher yield. The lowest percentage of potash in the leaf extract occurred in the check plots, followed by no-potash plots. Yields showed the same sequence.

5. No-potash plots showed a higher content of potash in the leaf extract than did the check plots; indicating that the addition of nitrogen and phosphorus alone stimulated the plant to absorb more potash from the soil.

6. The percentage of phosphorus in the alcoholic leaf extract was not correlated with the amount of this nutrient applied in the fertilizer. On the other hand, it showed a marked parallelism with the amount of potash present in the alcohol-soluble portion of the leaves. The percentage of phosphorus in the alcoholic leaf extract was always low when the content of potash in the leaf was low, irrespective of the content of phosphorus in the fertilizer.

7. The percentage of nitrogen in the alcohol leaf extract did not present any correlation with the content of potash in the fertilizer.

8. The percentages of both calcium and magnesium in the leaf extracts showed an inverse relation to that of potash. They displayed a tendency to increase as the latter decreased and *vice-versa.*

9. The percentage of total nutrients in the mineral portion of the leaf extract (sum of $K_2O + P_2O_5 + N + Ca + Mg$) varied directly with the fertilizer treatment. In check and no-potash plots this value was lower than in plots receiving complete fertilizers.

10. The yield of potatoes in this investigation appeared to be correlated with the mineral composition of the dry alcoholic extract of the leaves. Both quantitative and qualitative relationships were shown, since the highest yielding plots showed a high mineral content and practically a definite ratio between the three major fertilizing nutrients; the lower yielding plots showed a lower mineral content and the ratio in which the

fertilizing elements were present departed from that found in the highest yielding plots.

Analyses for nitrogen, phosphorus, potash, calcium, and magnesium were run in duplicate by MR. J. MITCHELL of PURDUE UNIVERSITY under the direction of DR. H. R. KRAYBILL who volunteered to cooperate in this investigation.

MANZANA DE GOMEZ 516
HAVANA, CUBA

LITERATURE CITED

1. BARBIER, G. The mineral nutrition of the plant as a function of the chemical composition of its medium. Ann. Agron. (N.S.) **6**: 568–586. 1936.
2. BEAUCHAMP, C. E. and LAZO, F. The composition of the crude chlorophyll of cane leaves and its relationship with cane growth. Proc. of the Twelfth Annual Conference of the Association of Sugar Cane Technologists of Cuba. Pp. 34–47. December, 1938.
3. ——————, ——————, and BONAZZI, A. Studies in sugar cane physiology. V. The content of nitrogen, phosphorus, and potash in crude chlorophyll and in the leaf skeleton and its relationship with fertilizers. Proc. of the Eighth Annual Conference of the Assoc. of Sugar Cane Technologists of Cuba. Pp. 82–114. December, 1934.
4. ——————, ——————, and ——————. Studies on the physiology of sugar cane. VI. The content of nitrogen, phosphorus, and potash in fertilizers and their relationship with the composition of the crude chlorophyll of the leaves and the growth of the cane. Proc. of the Ninth Annual Conference of the Association of Sugar Cane Technologists of Cuba. Pp. 26–37. December, 1935.
5. CAROLUS, ROBERT L. Effect of certain ions, used singly and in combination, on the growth and potassium, calcium, and magnesium absorption of the bean plant. Plant Physiol. **13**: 349–363. 1938.
6. ——————. The relation of potassium, calcium, and sodium to magnesium deficiency. Proc. Amer. Soc. Hort. Sci. **33**: 595–599. 1939.
7. COOPER, H. P. Relation of lime application to availability of potash in soil. South Carolina Agr. Exp. Sta., 47th Ann. Rep. p. 29. 1934.
8. DELBERT, PIERRE. Agriculture, kitchen, and magnesium. Bull. Acad. Méd. **111**: 393–415. 1934.
9. EHRENBERG, PAUL. Quoted by E. J. RUSSEL. Soil conditions and plant growth, 5th edition, p. 100. 1927.

10. HARDY, F., McDONALD, J. A., and RODRIGUEZ, G. Leaf analysis as a means of diagnosing nutrient requirements of tropical orchard crops. Jour. Agr. Sci. **25**: 610–627. 1935.

11. LAGATU, H. and MAUME, L. The variations of the sum $N + P_2O_5 + K_2O$ per 100 parts of dry material of the leaf of a cultivated plant. Compt. Rend. Acad. Agr. France **21**: 85–92. 1935.

12. ———— and ————. Leaf diagnosis of tobacco: Comparative effects of basic slag, superphosphate, and basic phosphate of the nitrogen-phosphorus-potassium equilibrium. Compt. Rend. Acad. Sci. (Paris) **200**: 502–504. 1935.

13. ———— and ————. Qualitative and quantitative variations in the NPK nutrition of the same species of the plant in the same soil without fertilizer interference. Prog. Agr. **105**: 353–356. 1936.

14. ———— and ————. The sensitivity of foliar diagnosis for revealing fertilizer absorption by the vine (grape). Compt. Rend. Acad. Agr. France **24**: 615–624. 1938.

15. LESCH, W. The influence of potash fertilization upon the ratio of bases in hay. Zeitschr. Pflanzenernährung, Düngung & Bodenk. **13B**: 211–225. 1934.

16. LOWRY, M. W., HUGGINS, W. C., and FORREST, L. A. Effect of soil treatment on the mineral composition of exuded maize sap at different stages of development. Georgia Agr. Exp. Sta. Bull. 193. 1936.

17. McCALLA, A. G., and WOODFORD, E. K. The effect of potassium supply on the composition and quality of wheat. II. Canadian Jour. Res. **13C**: 339–354. 1935.

18. McINTIRE, W. H., SHAW, W. M., and YOUNG, J. B. The repressive effect of lime and magnesia upon soil and sub-soil potash. Jour. Agr. Sci. **20**: 499–510. 1930.

19. MAUME, L., and DULAC, J. The important rôle of the medium (climate and soil) in the absorption of nitrogen, P_2O_5 and K_2O by wheats which have reached the same physiological period. Compt. Rend. Acad. Agr. France **21**: 120–123. 1935.

20. SHIBUYA, K., and TORII, TAKASHI. The antagonistic action between potash and alkaline earths for plants growth. Jour. Sci. Soil and Manure (Japan) **9**: 411–424. 1935.

21. SÜCHTING, H., JESSEN, W., and MAURMANN, G. The action of fertilizers applied to forest soils. II. Testing the activity of nutrients by physiological methods. Bodenk. u. Pflanzenernähr. **8**: 272–335. 1938.

22. THOMAS, W. Foliar diagnosis: Principles and practice. Plant Physiol. **12**: 571–599. 1937.

23. ————. Foliar diagnosis: Its relation to the optimum nutrition of the potato. Plant Physiol. **13**: 677–694. 1938.

24. ————, and MACK, W. B. Foliar diagnosis: Physiological balance between the bases lime, magnesia, and potash. Plant Physiol. **14**: 699–716. 1939.

25. YOSHIAKI, ISHIZUKA I. Calcium-potassium law of Ehrenberg. Jour. Agr. Chem. Soc. (Japan) **11**: 574–588. 1935.

SOME ASPECTS OF MINERAL NUTRITION IN RELATION TO BISON FLAX

E. A. Helgeson, T. H. Hopper, and David Taylor

(WITH THREE FIGURES)

Introduction

That the yield and quality of oil produced by flax seed varies from year to year is a matter of common knowledge. In the absence of exact experimental data it is frequently stated that climatic factors are responsible for this variation. Since little is known about the actual process of oil synthesis by the flax plant and still less about the effect of specific environmental factors on this synthesis, it was felt that some careful studies under controlled conditions should be initiated. In these first studies the authors undertook to grow seed flax in selected nutrient solutions in an attempt to determine if there was any relation between mineral nutrition and the quality and quantity of the oil produced from flaxseed.

Fertilizers and their relation to yield of flax grown in the field have received some attention by various workers throughout America; little exists in the literature, however, relative to the growth of flax in nutrient solutions. Turner (15) studied the effect of variations in the nitrogen supply on the ratio of top to root growth of Winona flax. Using solutions high and low in nitrate nitrogen, he found that there was little difference in the nitrogen content of flax grown in these nutrients. He attributed the results obtained to relatively limited use of nitrogen by flax, and concluded that in many cases nitrogen fertilizers when applied to flax are not particularly beneficial.

After studying the effect of the light factor on cell production of fiber flax grown in sand culture, Robinson (8) reported that the tallest growth resulted when fiber flax was grown in ten hours of light per day; flax which was exposed to light eighteen hours each day matured earliest but produced the least vegetative growth. Thus the growth period of flax might be shortened by lengthening the photoperiod. His report showed that for fiber flax nitrogen was particularly important in early growth, and that potassium was more important as the plants matured. Adams (1) worked out the relation between duration of light and growth, with reference to flax grown in soil, and his findings in most instances bear out those of Robinson.

In Germany, Selle (11) made tests on soil acidity in relation to growth of fiber flax. He noted that the yields, based on fiber, were greatest on lime-rich soils and poorest on acid soils. Potassium, according to Powers (7), was of the greatest importance in fertilizers for fiber flax; its presence

in the soil increased strength and length of fiber and vigor of growth. Nitrogen was second in importance. Potash was required relatively early in order to obtain maximum benefits.

Fiber flax was grown in liquid nutrient media by SHKOL'NIK (12). He worked especially with boron, manganese, aluminum, and copper. Boron was found to be of paramount importance, its absence causing a poor development of the root system and eventual death. The optimum concentration of boron was 0.5 mg. per liter; doses of about 5 mg. per liter were toxic. Manganese and other elements mentioned appeared less important. A few years ago SOMMER (13) showed that the addition of small quantities of copper to nutrients supporting flax growth resulted in increased vigor as compared to growth of plants in solutions lacking that element.

SCHMALFUSS (9) grew fiber flax in pots of loamy soil, the water content of which was controlled, and which received various fertilizer treatments. He reported the following findings: lignification of fiber cells was increased by lowering the water content of the soil, and also by increasing the amount of sulphate ion added; lignification was decreased by increasing the amount of chloride ion; the fiber content of the stem was increased by nitrogen, and more so by K_2O; the fiber content was increased by the sulphate ion, and decreased by the chloride ion. He further reported that the yield and quality of oil produced were affected by fertilization. In this respect the iodine number of oil from nitrogen-starved plants was highest. The iodine number was affected more by anions than by cations; the chloride ion increased and the sulphate ion decreased the iodine number of the oil produced. His results showed that the nitrogen content of the seed varied inversely with the oil content. The observed reactions on amount and quality of fiber and oil he explained as results of effects of the above mentioned factors on the colloids, hydration of the plant cells, and the water economy of the plants.

No attempt will be made here to present an extended review of the literature on the subject of physiological nutrient solutions or on the technique of nutrient culture work. TOTTINGHAM (14) gave a thorough review of the subject in his paper of 1914, and since then a number of workers have dealt with various other phases. The triangle system as used in this work has been adequately discussed by SCHREINER and SKINNER (10) and MILLER (6).

Preliminary experiments were carried out in the winter of 1935–36 in order to find the approximate concentration of nutrients that would support the growth of flax. In these tests a pure line of Bison flax, developed by H. L. BOLLEY and O. HEGGENESS of the North Dakota Agricultural Experiment Station was grown to the flowering stage in SHIVE's type I, three-salt, liquid nutrient solutions. The best growth was obtained in solutions

having a composition of 25 to 37.5 per cent. KH_2PO_4, 50 to 62.5 per cent. $Ca(NO_3)_2$, and 25 to 37.5 per cent. $MgSO_4$, based on the total salt concentration of the nutrient solution.

Methods and materials

During the winter of 1936–37 Bison flax was grown to maturity in ten of SHIVE's type I, three-salt liquid nutrient solutions. These solutions were chosen so as to be well distributed over the triangle; they included solutions R1S5, R2S1, R2S3, R2S4, R2S5, R3S2, R3S3, R4S2, R4S3, and R5S2. Partial volume molecular concentrations are given in table II.

The necessary elements for plant growth are supplied in type I, three-salt solutions by KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$. In making up the nutrients these salts were added from M/2 stock solutions to distilled water in the proportions prescribed by the committee of Biology and Agriculture of the National Research Council in its 1919 report. The osmotic values of all solutions were determined cryoscopically and were found to be somewhat under one atmosphere.

Equal amounts of iron, manganese, and boron were added to each nutrient solution. The iron was added as soluble ferric phosphate from a 0.0134 M stock solution, 2 ml. of this solution being added to each liter of nutrient. One-half part per million of $MnSO_4$ and H_3BO_3 was added to each liter of nutrient solution prepared. All chemicals used in preparing the nutrient solutions were of C. P. analyzed grade secured from the J. T. Baker Chemical Company. Fresh solutions were prepared and added to the cultures daily.

A constant renewal system (fig. 1) was set up and regulated to feed, at the rate of 1.5 liters of solution per day, into each series of cultures. In this system the fresh nutrient was added to the upper reservoir, and from there it passed through a constant-level siphon, into the lower reservoir. The nutrient then flowed through the delivery siphon, dropped into the intake tube, and flowed down the tube into culture A. When the level of solution in culture A exceeded that in the delivery arm of the siphon, the solution was siphoned from culture A into culture B; the same process occurred in cultures B and C until the nutrient solution passed from that set of cultures into the drain. Each drop of nutrient, upon falling into the delivery tubes, would trap an air bubble, which was driven down through the intake tube, aerating the nutrient solution as it rose to the surface in each culture jar. A shield of insulation board was constructed to cover the reservoirs in order to prevent heating of the solutions in the reservoirs by the sun's rays. The term "bank" has been used to designate the three culture jars of a series. The two one-gallon reservoirs of each bank were closed with rubber stoppers and painted to exclude light, as was all glassware used in the set-up.

The paraffined stoppers, used to support the plants, were anchored in tin, two-quart Economy jar covers which were heavily coated with paraffin. Ten such banks, each composed of two reservoirs and three cultures, were used in the experiment. These banks remained in a fixed position during the entire study.

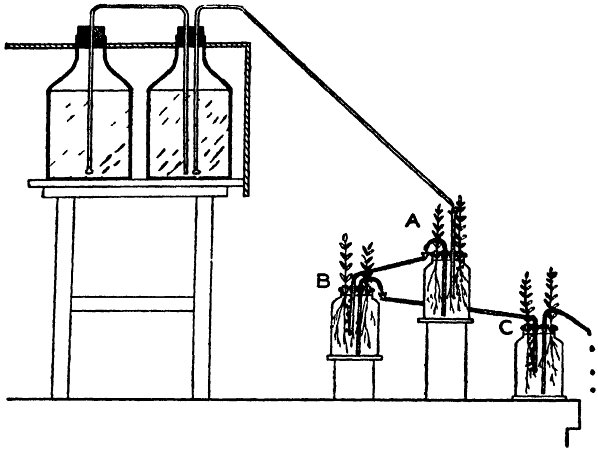


FIG. 1. Constant renewal system used in flax nutrition studies.

Seed of the Bison variety from the same lot used in the 1935–1936 studies was used in this experiment. Quantities of seedlings were obtained using methods similar to those described by HOAGLAND and BROYER (4). When the tops of the seedlings were 3 cm. long, uniform seedlings were selected, their stems were wrapped in cotton at the base and enclosed in a cork. Five seedlings were placed in each culture container. Cultures were set up on November 27, 1936. The plants were supported by a network of threads. The temperature of the greenhouse was thermostatically controlled at 18 to 20° C.; the relative humidity generally ranged from 40 to 50 per cent.

Throughout the experiment the plants were exposed to 24 hours of light each day. At night light was supplied by four 200-watt Mazda bulbs equipped with porcelain reflectors. As measured by the Weston Photronic cell a light intensity of from 130 to 140 foot-candles was obtained all along the table at the tops of cultures of each bank.

Hydrogen ion determinations were made on the freshly prepared nutrient solutions and the solutions leaving the different banks on three different days during the period of the experiment using both the Youden quinhydrone cell and colorimeteric procedures. In all cases these tests were conducted according to the directions given by CLARK (3).

All cultures were allowed to grow to maturity or until the main stems of the plants had completely dried. Upon removal of the plants from the

cultures, data on oven-dry weight of roots and tops were taken on the five plants from each culture. Dry weights were determined on material dried to constant weight at 70° C. After the seeds had been removed, fruits were hand picked and threshed, the seed from the main stems being kept separate from that of the lateral branches. A record was made of main stem, lateral, and total seed in each culture; and the weight of one hundred seed samples of the main stem seed was also recorded.

The flaxseed samples were stored togther with a bulk sample and all assumed to acquire the same moisture content as the bulk sample. Moisture determinations were run on the bulk sample since the experimental seed lots were too small to permit of such determinations. Each entire experimental lot was then weighed and thoroughly ground with round sand in a porcelain mortar. The ground seed and sand was carefully washed with Skelly Solve F into a special glass extraction thimble and extracted for 24 hours in a percolation extractor with the same solvent. The quantity of oil was determined by direct weighing of the oil in the tared extraction flask after removal of the solvent and drying in a vacuum oven for one half hour at 110° C. The iodine number of the extracted oil was determined refractometrically according to the method of HOPPER and NESBITT (5).

Total nitrogen was determined on the extracted residue by the official A.O.A.C. method.

Results and discussion

The hydrogen ion concentrations of the nutrient solutions were determined on freshly prepared solutions on January 17, February 28, and March 26. All of the fresh nutrients had pH values between 5.7 and 6.0. Likewise, the solutions coming out of the different culture jars were tested

TABLE I

CHANGES IN HYDROGEN ION CONCENTRATION OF NUTRIENT SOLUTIONS BROUGHT ABOUT BY THE GROWTH OF FLAX

SOLUTION AND DATE OF TEST	pH VALUE*									
	NUTRIENT NUMBER									
	R1S5	R2S1	R2S3	R2S4	R2S5	R3S2	R3S3	R4S2	R4S3	R5S2
Fresh solution	6.05	5.85	5.85	5.75	5.85	5.75	5.75	5.75	5.75	5.65
Used solution 1/17/37	5.9	5.7	5.75	5.75	5.75	5.65	5.6	5.65	5.6	5.65
Used solution 2/28/37	5.35	5.35	5.45	5.45	5.65	5.65	5.45	5.25	5.55	5.55

* Average of three cultures in bank.

on January 17, and February 28, fifty-one and seventy-five days, respectively, after the seedlings had been placed in the culture jars (table I). At the time the used solution was first tested, the plants were just entering the period in which they made most marked and rapid growth. The pH of solutions coming from the different cultures of a bank was uniform, and showed little change from that of the freshly prepared nutrients. The second test was made after the plants had attained a considerable size and were still growing rapidly. The results in the second test showed that the pH of the nutrients varied slightly as they left the different cultures of a bank; there was a general tendency, however, for the solutions to become slightly more acid as they flowed through the banks.

These changes seem to be in no way correlated with the original composition of the nutrient solutions. All solutions were uniform in that they tended to become more acid. CHIZHERSKAIA (2) carried on water culture experiments to determine the relation of sprout growth in flax to the pH of the nutrient medium. From these experiments she found that the maximum sprout growth occurred at pH 5, and pH 9. It must be remembered that her conclusions on acidity relations were based upon the early growth of flax, while in the experiments reported above large plants were involved.

Considering the nutrients on the basis of the length and dry weight of tops produced, it is evident that a slightly greater growth resulted in cultures containing relatively high concentrations of KH_2PO_4 (table II). In general there was no region of the triangle used where nutrients were definitely superior in respect to height and dry matter produced. There is no indication of a clear cut correlation between the composition of the nutrient solution and the final length of top growth; the cultures which were slowest to mature, however, R1S5 and R5S2, made the tallest top growth. Plants in all cultures were considerably taller than field grown plants of the same variety. According to the records available in this Experiment Station, this same strain, over a period of years, has produced plants ranging from 40 to 61 centimeters in height (data obtained from H. L. BOLLEY and O. HEGGENESS). The data on dry-matter produced can be considered only as relative owing to the fact that dry-weights could not be taken until the plants had matured, with a consequent loss of some leaf material. Yields in regard to dry-weight were rather definitely related to the concentration of KH_2PO_4, the largest amount of dry-matter being obtained in solutions with the highest concentration of that salt. Concentration of other salts in these high KH_2PO_4 cultures varied considerably. The data appear to indicate that this variety of flax is not a heavy user of nitrogen.

The date of maturity of plants growing in the different solutions showed a certain degree of relationship to the amount of KH_2PO_4 present. Those solutions relatively high in KH_2PO_4 produced the earliest maturing plants.

TABLE II

COMPOSITION OF SELECTED SHIVE'S TYPE I NUTRIENT SOLUTIONS, VEGETATIVE GROWTH, SEED PRODUCTION, AND CHEMICAL ANALYSIS O[F] SEEDS PRODUCED IN THESE SOLUTIONS

Partial volume molecular concentrations of solutions used					Data on vegetative growth and seed production*					Data from chemical analysis			
												Percentage crude protein ($N \times 6.25$)	
Bank No.	Sol'n No.	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Growth period	Height of top	Dry matter per plant**	Average total seed per plant†	Average weight per 100 main-stem seeds	Percentage oil content	Iodine number	Meal basis‡	Oil and water free basis
					days	*cm.*	*gm.*	*gm.*	*gm.*	%		%	%
1	R1S5	0.0022	0.0108	0.0043	185	119.4	2.566	0.428	0.641	36.6	164	27.6	47.74
2	R2S1	0.0053	0.0027	0.0132	169	106.7	2.267	0.519	0.633	36.7	174	25.7	44.63
3	R2S3	0.0047	0.0071	0.0071	159	109.2	2.378	0.611	0.663	37.2	174	25.3	44.27
4	R2S4	0.0045	0.0090	0.0045	161	95.3	1.724	0.453	0.655	37.9	175	24.8	43.88
5	R2S5§	0.0041	0.0104	0.0021	149	97.8	1.487	0.342	0.636	37.2	175	27.9	48.84
6	R3S2	0.0072	0.0048	0.0072	149	111.8	2.249	0.695	0.672	37.7	181	24.8	43.86
7	R3S3	0.0068	0.0068	0.0045	177	99.0	2.899	0.719	0.661	36.1	172	26.7	45.85
8	R4S2	0.0094	0.0047	0.0047	177	106.7	3.037	0.697	0.686	36.6	175	26.9	46.67
9	R4S3	0.0090	0.0068	0.0022	174	109.2	2.927	0.655	0.660	36.3	177	26.9	46.49
10	R5S2	0.0118	0.0047	0.0023	183	120.7	3.248	0.672	0.662	36.1	174	27.1	46.58

* Average value per plant from 15 plants in all three cultures of each bank.
** Exclusive of chaff.
† Total lateral and main-stem seed.
‡ Moisture assumed at 5.68% on basis of other samples stored under same condition and analyzed.
§ Figured on basis of culture 1 only.

Plants in high-nitrogen cultures tended to be more vegetative. Cultures high in KH_2PO_4 and relatively high in nitrogen were intermediate as regards maturity. Magnesium sulphate seems to have played a less positive rôle as far as length of growth period is concerned. The character of growth is shown in figures 2 and 3.

FIG. 2. The appearance of the top growth in the cultures of banks 1, 2, and 3 on January 1, 1937.

From the standpoint of total weight of seed produced, solutions medium to high in KH_2PO_4 again showed the best yields (table II). The presence of medium to high concentrations of the nitrogen-carrying salt seemed to depress the total yield of seed unless the amount of acid phosphate present was also fairly large. Data obtained on the yield from plants growing in the field showed those plants had produced, over a period of years, about 1 gram of seed per plant. In this experiment the yields in the different cultures ranged from 0.[illegible]42 to 0.719 gm. per plant which was considerably less than the yields obtained from field grown flax of this variety.

The weight of 100 average seeds produced on the main stems of the plants in the different nutrient media ranged between 0.633 and 0.686 gm. Seeds from a large number of samples of Bison flax that were grown in the

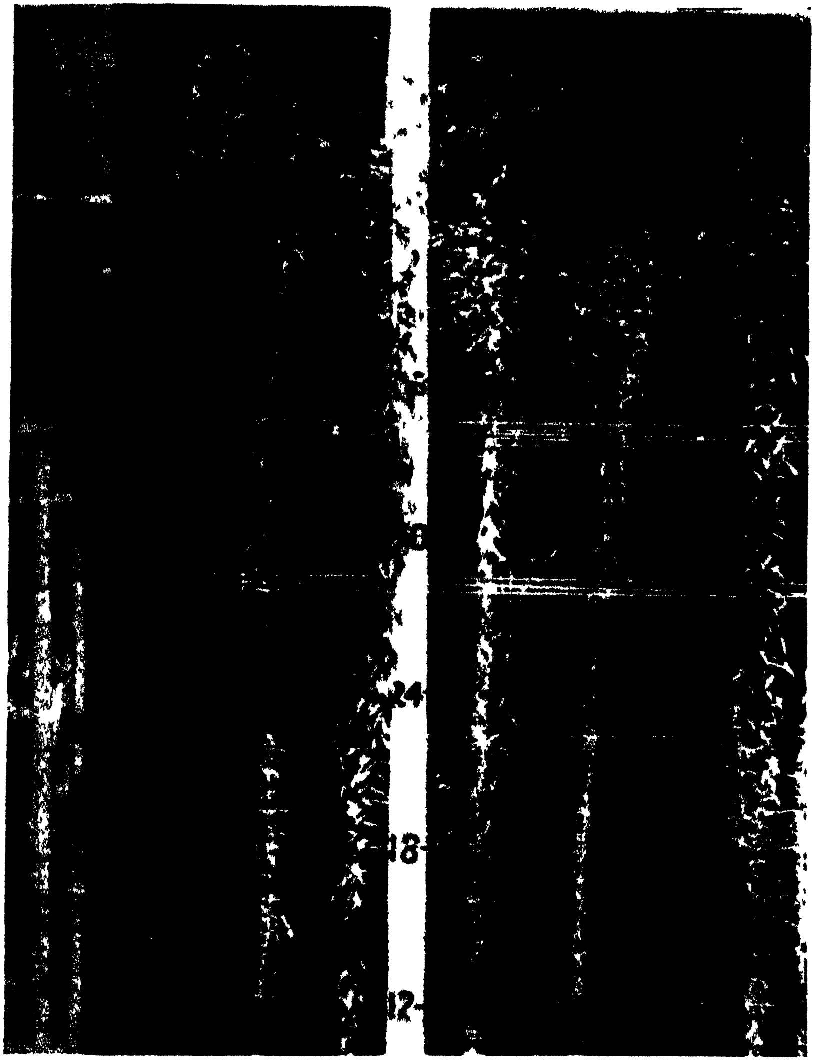


FIG. 3. The appearance of the top growth in the cultures of banks 1 and 2 on March 25, 1937.

field in different localities varied from 0.318 to 0.677 gm. per 100 seeds and averaged about 0.533 gm. per 100 seeds. Our seed with an average of 0.657 gm. per 100 seeds was generally heavier than seed from this variety grown on plants in the field. The general tendency was toward heavier seed in cultures relatively high in KH_2PO_4; whereas plants grown on the nitrogen-high nutrient tended to produce lighter seed. All seed produced was, however, surprisingly uniform in weight (table II).

Analysis of the seed produced during this study showed that the yield of oil was about equal to that of the average field-grown seed, and that the iodine number of the oil was comparable to the highest of oil produced in field-grown flax at this station (table II). The amount of oil produced with the different nutrient solutions was rather constant, varying from 36.1 to 37.9 per cent., when calculated on a 5.68 per cent. moisture basis. Commercial samples of Bison varied from 32.0 to 40.0 per cent. with an average of 37.0 per cent. when calculated on the same basis. Tests for iodine number, made on the seed harvested from plants grown in this experiment, gave a range of from 164 to 181. Oil having the highest iodine number was produced in cultures well supplied with potassium acid phosphate. Data from the records on field tests conducted at Fargo show oils from flax-seed produced on Bison flax grown in the field had iodine numbers of 161 to 167. These findings indicate that the degree of saturation of linseed oil is not a fixed character but varies with the nutrient supply available.

The protein content of the seeds from the main stems of plants ran uniformly high in all cases, ranging from 38.36 to 49.63 per cent, on an oil and water free basis. Other experimental samples from field grown material have been found to vary from 28.4 to 53.4 per cent. crude protein on the same basis. Cultures high in $Ca(NO_3)_2$ and fairly high in KH_2PO_4 produced the highest yields of protein although the total difference was not great. Considering the yield of oil in relation to the protein content in the seeds one finds a fairly definite inverse relationship. It will be seen from table II that in some cases the protein content of the seeds decreased upon increase in oil content. This correlation bears out the findings of SCHMALFUSS (9) regarding the relation between nitrogen fraction and oil content.

Summary

In this study a pure line of Bison flax was grown to maturity in selected SHIVE's type I, three-salt nutrient solutions using a constant renewal technique. Temperature, humidity, and light conditions under which the plants grew were recorded. The data obtained on plants grown under the conditions of this experiment indicate that:

1. Final growth in height of top is relatively constant over a wide range of nutrient salt proportions.

2. The presence of fairly large quantities of KH_2PO_4 in the nutrient solutions resulted in relatively early maturity, the date of maturity being modified by the nitrogen content of the nutrient solution. The time of maturity seemed to be in inverse proportion to the amount of nitrogen present.

3. Relatively greater amounts of oven-dry material were obtained with nutrient solutions high in KH_2PO_4. The other constituent salts of the

various nutrient solutions showed no uniform relation to the oven-dry weight of plant material produced.

4. Generally the greatest seed production occurred in solutions containing the higher proportions of KH_2PO_4; other nutrient constituents show no definite relation to this criterion. The heaviest seed was obtained from plants which grew on nutrients relatively high in the potassium acid phosphate salt.

5. There is no marked variation in quantity of oil produced when the composition of the nutrient solution is varied over a rather wide range.

6. Oils from seeds produced in cultures relatively high in KH_2PO_4 have higher iodine numbers than those from cultures low in this salt.

7. The production of plants grown on nutrient solutions in constantly renewed solution cultures exceeded production of field-grown plants in the following respects: height of top, weight of seed, average protein content of seed, and iodine number of oil. The oil yields in the experimental plants about equal average oil yields of field grown plants.

North Dakota Agricultural Experiment Station
Fargo, North Dakota

LITERATURE CITED

1. Adams, J. Duration of light and growth. Ann. Bot. **38**: 509–523. 1924.
2. Chizherskaia, Z. A. Physiological studies of flax. (In Russian with English summary.) Mem. Inst. Agron, Leningrad **4**: 253–288. 1927.
3. Clark, W. M. The determination of hydrogen ions. 3rd ed. Williams and Wilkins Company, Baltimore. 1928.
4. Hoagland, D. R., and Broyer, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
5. Hopper, T. H., and Nesbitt, L. L. The relations between refractive index and iodine number of raw linseed oil. Oil and Soap **14**: 34–36. 1937.
6. Miller, E. C. Plant physiology. McGraw-Hill Book Company, New York. 1931.
7. Powers, W. L. Fertilizers for fiber flax. Jour. Amer. Soc. Agron. **20**: 755–763. 1928.
8. Robinson, B. B. Some physiological factors influencing the production of flax fiber cells. Jour. Amer. Soc. Agron. **25**: 312–328. 1933.
9. Schmalfuss, K. Experimental studies on the physiology and nutrition of flax with respect to formation of fiber and oil. I. Pot Expts. Bodenk in Pflanznernähr. **1**: 1–39. 1936.

10. SCHREINER, O., and SKINNER, J. J. Ratio of phosphate, nitrate, and potassium on absorption and growth. Bot. Gaz. **50**: 1–30. 1910.
11. SELLE, H. Die Bedeutung der Bodenazidität für das Flachswachstum. Faserforchung **5**: 146–152. 1926.
12. SHKOL'NIK, M. Effect of boron and other additional elements on the development of flax (in Russian). Bull. Acad. Sci. USSR. Cl. Sci. Math. Nat. **8**: 1163–1188. 1933.
13. SOMMER, A. L. Copper as an essential element for plant growth. Plant Physiol. **6**: 339–345. 1931.
14. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant culture. Physiol. Res. **1**: 133–245. 1914.
15. TURNER, T. W. The effect of a varying nitrogen supply on the ratios between tops and roots in flax. Soil Sci. **21**: 303–306. 1926.

AUXITHALS SYNTHESIZED BY SOME FILAMENTOUS FUNGI[1]

LEON H. LEONIAN AND VIRGIL GREENE LILLY

Introduction

Since 1869 when RAULIN reported that *Aspergillus niger* excreted some growth-promoting substances into its medium, a number of investigators have studied the phenomenon of auxithal synthesis by fungi. For a comprehensive review of this the reader is referred to FRIES (2) and SCHLENKER (7). The writers (5) have referred briefly to this subject; the present paper reports a more exhaustive study.

The following six organisms were used as sources of auxithals: ***Rhizopus suinus, Fusarium niveum, Phytophthora erythroseptica, Phycomyces blakesleeanus, Pythiomorpha gonapodioides***, and ***Mucor ramannianus***. The first two fungi thrive in synthetic media without the addition of any growth substance; ***Phytophthora erythroseptica*** cannot grow without thiamin; ***Phycomyces blakesleeanus*** grows either in the presence of thiamin or of a mixture of pyrimidine and thiazole (thiamin moieties). ***Pythiomorpha gonapodioides*** grows in the presence of pyrimidine alone as well as in the presence of thiamin; ***Mucor ramannianus*** requires only thiazole for growth, but it does very well in the presence of thiamin.

Materials and methods

The nutrient solution employed by the writers consisted of the following: 0.5 gram each of ammonium nitrate, magnesium sulphate and potassium dihydrogen phosphate, ROBBINS and KAVANAGH's (6) modification of HOAGLAND's A–Z mixture of rare elements, 1 gram of a mixture of amino acids (d-arginine, d-glutamic acid, l-aspartic acid two parts each; dl-a-alanine, and glycine one part each), 5 grams Bacto dextrose, and 1000 ml. distilled water. The pH of the medium was adjusted to 5.5 with sodium hydroxide. Thiamin or its moieties were used at the rate of 1:20 million. The medium in which ***Rhizopus suinus*** and ***Fusarium niveum*** were grown contained neither thiamin nor its moieties; ***Phytophthora erythroseptica*** was grown in the presence of thiamin, ***Phycomyces blakesleeanus*** in the two moieties, ***Pythiomorpha gonapodioides*** in pyrimidine only, and ***Mucor ramannianus*** in thiazole only.

Fernbach flasks of 2800-ml. capacity were used for the cultures; each flask contained 500 ml. of the nutrient solution. The fungi were allowed to grow at room temperature and in diffused light until a mycelial mat formed on the surface of the medium. ***Phytophthora erythroseptica*** required 25

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 235.

days to reach this stage of development, *Mucor ramannianus* and *Fusarium niveum* two weeks, and the others 10 days.

The mycelium was filtered off, well washed with water, air dried at 30° C., extracted with water by autoclaving ten minutes at 15 pounds pressure, filtered, taken to dryness *in vacuo,* and added to the basic medium at the rate of 0.1 per cent. The filtered medium was treated with activated charcoal (Norit), eluted with pyridine-water (2:1), the solvent removed under diminished pressure, and the residue added to the basic medium at the rate of 0.1 per cent. Two per cent. agar was added to the medium which was then adjusted to pH 5.5, tubed, sterilized, and inoculated. After growing for two weeks at 25° C. the readings were taken.

Forty fungi were used to determine the auxithals present in the mycelium and the medium of the six fungi. Twenty-four of these organisms can grow only when thiamin or its moieties are added to the basic medium, while the remaining 16 do not respond significantly to thiamin or its moieties but must have biotin. The writers do not wish to convey the impression that thiamin or its moieties alone, or biotin alone, induce *optimum* growth in their respective groups of fungi because other chemicals and physical factors also may be accessory. For instance, *Acanthorhynchus vaccinii* grows fairly well in the presence of thiamin alone but produces a much richer growth when biotin is added; *Ashbya gossypii* makes only a slight growth when thiamin, biotin, and inositol are used separately, but when these three are added to the same medium a rich growth follows. Furthermore, organic acids, mixtures of different sugars and of nitrogen compounds, reducing substances, surface area and depth of the medium, higher concentrations of foods, etc. greatly influence the amount of growth. Organic compounds contain other and as yet unknown substances that bring about increased growth. For instance, grains, fungus extracts, and animal excreta induce growth increases far beyond the limits of their thiamin and biotin content.

The term biotin is used here reservedly as we have only circumstantial evidence that the specific auxithal responsible for the growth of certain organisms is biotin. Kögl and Tönnis (3) thus far are the only investigators to have obtained biotin in pure form. Kögl and Fries (4) found that *Ashbya gossypii* and *Lophodermium pinastri* need biotin among other things before they can grow on synthetic media. The organisms listed by the writers as requiring biotin respond to the same auxithals as *Ashbya gossypii* and therefore are tentatively listed as organisms responding to biotin.

Discussion

Table I gives the response of biotin-requiring organisms. Roman numerals indicate the relative amount of growth; thus, 0 indicates no growth; I and II, trace; III, slight; IV and V, poor; VI and VII, fair; VIII, good;

X, excellent. A number of organisms can make a thin, submerged growth in the absence of auxithals, while upon the addition of such substances they grow profusely and the aerial mycelium fills the test tube.

The foregoing table (table I) shows that all six of the test fungi are capable of synthesizing biotin, a larger part of which is retained in the mycelium but considerable quantities diffuse into the medium. *Mucor ramannianus* stores up very small amounts of biotin and gives off into the medium still smaller quantities.

Organisms that are dependent upon extraneous sources of biotin require different amounts of it for optimum growth. For instance, *Sordaria fimicola* grows and reproduces very readily in the presence of the extract of *Fusarium niveum*; whereas *Allomyces javanicus* makes a slight growth; in fact it does not grow well in the presence of any of the extracts. It is possible, however, that the factor which limits the growth of this fungus may not be biotin but some sugar or some nitrogen compound. Our strain of *Saccharomyces cerevisiae* responds well to minute amounts of biotin while many others do not.

Because the hyphal and mycelial extracts used here are complex substances and contain, in addition to biotin, inositol, and thiamin, many other compounds some of which may act as auxithals, it will not be possible at this time to determine more closely the specific responses of different fungi to biotin alone.

Synthesis of thiamin by the six test fungi

The 24 organisms listed in table II require thiamin for growth. When transferred to the basic medium containing neither thiamin nor its moieties, they make either no growth, or form very sparse, submerged hyphae. Upon the addition of crystalline thiamin they all thrive and produce a rich aerial mycelium. Since the various fungal extracts replace thiamin, as can be seen in the following table, and no other known compound can bring about growth, it is assumed that the growth-promoting substance present in the mycelium of the six fungi tested consists of thiamin.

Table II shows that the extract from the medium is a poorer source of thiamin or its moieties than is the extract from the mycelium. Such organisms as *Lenzites saepiaria, Phytophthora erythroseptica, Pythiomorpha oryzae, Pythium ascophallon, Thielaviopsis basicola*, etc., that are unable to utilize thiazole and pyrimidine, alone or in mixture, but must have the thiamin molecule, failed to thrive in the medium extract of all six organisms. However, the organisms capable of utilizing thiazole and pyrimidine alone or in mixture made a good growth in the medium extract of all organisms except that of *Mucor ramannianus*. This indicates that the growing mycelium absorbs all the thiamin from the medium and retains it as in case

TABLE I

THE EFFECT OF THE MYCELIUM EXTRACT AND MEDIUM EXTRACT UPON THE GROWTH OF BIOTIN-REQUIRING ORGANISMS

ORGANISMS	*Rhizopus suinus*		*Fusarium niveum*		*Pythiomorpha gonapodioides*		*Mucor ramannianus*		*Phycomyces blakesleeanus*		*Phytophthora erythroseptica*	
	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT
Acanthorhynchus vaccinii	VIII	IV	VIII	VIII	VII	VII	V	III	VII	VII	X	V
Allomyces javanicus	V	0	IV	0	III	0	I	0	II	0	V	0
Ashbya gossypii	VI	0	VIII	I	VI	I	I	I	V	I	VII	I
Ceratostomella multiannulata	X	III	X	II	X	II	III	I	X	V	X	IV
Dipodascus uninucleatus	X	0	VIII	I	III	IV	III	III	VII	II	VIII	IV
Lophodermium pinastri	VII	II	VI	II	VII	II	II	II	VIII	II	X	II
Ophiobolus oryzinus	X	0	X	II	X	I	III	II	X	II	X	V
Ophiostoma catonianum	X	V	X	II	VI	II	II	II	X	II	V	0
Pseudopeziza ribis	VII	II	VII	II	V	IV	II	II	VII	II	V	II
Rosellinia arcuata	X	III	X	II	X	III	IV	II	X	II	X	VIII
Saccharomyces cerevisiae	VIII	VII	X	VIII	VII	VII	VI	VI	X	VII	X	VIII
Saccobolus depauperatus	VII	III	VII	III	VII	VI	IV	II	VII	IV	V	III
Sordaria fimicola	X	VII	X	X	VI	IV	X	IV	X	III	X	VIII
Sporormia intermedii	X	I	V	II	V	I	II	II	VIII	I	X	IV
Thraustoteca clavata	VII	I	VI	I	VI	I	V	I	VII	I	VII	I
Trachysphaera fructigena	VIII	VI	VII	VII	VII	VI	V	IV	X	VIII	VI	II

TABLE II

THE EFFECT OF THE MYCELIUM EXTRACTS AND THE MEDIUM EXTRACTS UPON THE GROWTH OF THIAMIN-REQUIRING ORGANISMS

Organisms	*Rhizopus suinus*		*Fusarium niveum*		*Pythiomorpha gonapodioides*		*Mucor ramannianus*		*Phycomyces blakesleeanus*		*Phytophthora erythroseptica*	
	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract	Hyphal extract	Medium extract
Blakeslea trispora	X	VIII	X	VIII	X	X	VIII	VI	X	X	X	X
Chaetocladium brefeldii	VI	II	VII	III	VII	IV	II	I	X	VI	X	X
Collybia tuberosa	VII	V	X	II	X	VII	II	I	X	X	VIII	VII
Coprinus lagopus	X	III	VII	II	VII	IV	II	I	VII	IV	VI	III
Coprinus turgiversans	VII	V	X	II	X	X	I	I	X	X	X	X
Cyathus striatus	VIII	V	VI	IV	VIII	VII	III	II	VII	VI	VII	IV
Deconica inquilina	VIII	II	VII	II	VIII	II	II	II	VIII	II	VII	II
Dermatia balsamea	V	II	IV	II	V	V	III	II	V	V	V	IV
Gloeocystidium roseo-cremeum	X	V	X	I	V	I	III	I	VIII	I	X	I
Lentinus tigrinus	X	VII	X	VIII	X	X	VIII	VII	X	X	X	X
Lenzites saepiaria	VIII	0	X	0	V	0	0	0	X	0	VI	I
Lenzites betulina	X	II	X	III	X	VIII	III	II	X	VIII	X	VIII
Mucor ramannianus	X	VIII	X	X	VIII	VIII	VIII	VII	X	X	X	X
Phycomyces blakesleeanus	X	I	X	II	X	IV	III	II	X	VIII	VIII	VI
Phytophthora erythroseptica	X	0	X	0	X	0	III	0	X	0	X	I
Phytophthora fagopyri	X	VI	X	IV	X	X	VII	V	X	X	X	X
Pilaira moreaui	X	II	X	II	IV	II	IV	II	X	VIII	X	IV
Pythiomorpha gonapodioides	X	V	X	IV	X	X	V	III	X	VIII	X	VI
Pythiomorpha oryzae	VI	0	VI	0	VI	I	I	0	VI	0	VIII	I
Pythium ascophallon	X	0	X	0	X	I	I	0	X	0	X	I
Pythium oligandrum	X	II	X	I	VII	II	I	I	X	IV	VIII	V
Pythium polymastum	X	0	X	0	VI	III	I	0	X	0	X	V
Thielaviopsis basicola	X	0	VI	I	VI	I	IV	I	VII	I	X	II
Typhula variabilis	X	VIII	X	X	X	X	VII	V	VI	VII	X	X

of *Phytophthora erythroseptica,* giving off into the medium the first disintegration products of thiamin—the thiazole and the pyrimidine compounds. In case of *Phycomyces blakesleeanus* the mycelium absorbs pyrimidine and thiazole from the medium, converts them into thiamin, some of which is stored, and the balance is used up. This utilization of thiamin by the fungus results in the production of pyrimidine and thiazole which diffuses into the medium. It is not probable that the thiazole and pyrimidine content of the extract of Phycomyces medium is the original material added to the solution because a demand on the part of the protoplasm of the mycelium for thiamin would eventually drain these substances from the medium just as *Phytophthora erythroseptica* drained all thiamin from its medium.

The following experiment was made in order to demonstrate this: thiamin was substituted for pyrimidine and thiazole in the nutrient solution which was inoculated with *Phycomyces blakesleeanus.* After a growth of 10 days at 25° C. the mycelium was filtered off and the medium treated and tested as before. It contained no thiamin as shown by the failure of *Phytophthora erythroseptica* to grow, but enough pyrimidine and thiazole to induce growth in *Phycomyces blakesleeanus, Pythiomorpha gonapodioides,* and *Mucor ramannianus.* Since *Phycomyces* is capable of synthesizing thiamin from the moieties, the thiazole and the pyrimidine given off into the medium would have to be absorbed sooner or later and utilized. This was found to be the case by the following experiment: the fungus was grown in flasks of 250-ml. capacity, containing 25 ml. of the medium plus 1/100 p.p.m. of the moieties. At regular intervals the mycelium was filtered off, the medium solidified with agar and tested. It was seen that eventually all the thiazole content of the medium was reabsorbed but some of the pyrimidine was left as shown by the failure of *Mucor ramannianus* to grow at all and by the ability of *Pythiomorpha gonapodioides* to grow. On the other hand, the mycelium will contain some thiamin so long as it lives. This was demonstrated by the following experiment: *Phycomyces blakesleeanus* was grown in the nutrient solution containing one part of thiamin in twenty million parts of the medium. Ten days later when the colony reached its maximum development, it was removed, washed with sterilized distilled water and transferred to a flask containing distilled water. One month later the mycelium was removed, washed in distilled water, transferred to a volume of distilled water and extracted by autoclaving. After the nutrients were added to this, sterilized and inoculated, it was found that *Phytophthora erythroseptica,* a thiamin-requiring organism, was induced to grow.

BONNER and ERICKSON (1) failed to obtain growth in thiamin-requiring *Phytophthora cinnamoni* when they added to their medium varying amounts of mycelium of *Phycomyces blakesleeanus* grown in a basic solution that contained a mixture of pyrimidine and thiazole. This appears to leave un-

answered the question of the ability of this species of Phycomyces to synthesize thiamin; yet table II shows that a number of thiamin-requiring fungi grew well in the presence of the mycelium extract of *Phycomyces blakesleeanus.* The cause of this discrepancy appears to lie in the Schopfer's medium used by BONNER and ERICKSON. While the abnormally high concentration of dextrose in Schopfer's solutions is readily utilized by a number of zygomycetous fungi, many of the Oomycetes, such as Phytophthoras, make no, or very little, growth in the presence of so much sugar. The small amount of thiamin present in the mycelium of *Phycomyces blakesleeanus* apparently was not enough to overcome the inimical effect of 10 per cent. dextrose.

Additional support to thiamin synthesis by Phycomyces is given by the following experiment: *P. blakesleeanus* was grown in the basic solution to which was added a mixture of pyrimidine and thiazole equivalent to 1/10th p.p.m. thiamin. Ten days later the mycelium was harvested, air dried, and varying amounts of it (at the rate of 1000, 250, 50, and 10 p.p.m.) were added to the basic medium. Twenty-five ml. of this nutrient solution was placed in a series of 250-ml. capacity flasks, sterilized and inoculated with *Phycomyces blakesleeanus* and *Phytophthora erythroseptica.* The organisms were incubated at 25° C. for 10 and 24 days respectively; the mycelium was then harvested and dried to constant weight at 100° C. Each figure in table III represents the aggregate weight of five cultures.

TABLE III

THE YIELD OF MYCELIUM INDUCED BY ADDING VARYING QUANTITIES OF DRIED MYCELIUM OF *Phycomyces blakesleeanus* TO THE MEDIUM

THE RATE OF DRIED MYCELIUM OF *P. blakesleeanus* ADDED TO THE BASIC MEDIUM	YIELD OF *P. blakesleeanus.* WEIGHT OF TOTAL MYCELIUM	YIELD OF *Ph. erythroseptica.* WEIGHT OF TOTAL MYCELIUM
p.p.m.	*mg.*	*mg.*
1000	211	325
250	199	273
50	113	271
10	72	28

The decrease in yield of mycelium, particularly in case of *Phytophthora erythroseptica,* is not proportionate. In a nutrient solution of low concentration, such as used in this work, the optimum amount of thiamin needed by this organism is extremely small and an amount of more than 1/1000th p.p.m. thiamin will exert little effect upon the yield. The significant fact brought out by the foregoing table is that *Phycomyces blakesleeanus* is capable of linking pyrimidine and thiazole to form thiamin as demonstrated by the fairly large yield of mycelium made by the thiamin-requiring *Phytophthora erythroseptica.*

Referring back to table II it should be noted that *Pythiomorpha gonapodioides,* unlike *Phycomyces blakesleeanus,* can synthesize its own thiazole which it links with pyrimidine to produce thiamin, retains the latter, and breaks down into its first disintegration products some of which escape into the medium.

Rhizopus suinus and *Fusarium niveum* are capable of synthesizing their own thiamin as well as biotin from pure dextrose, amino acids, and inorganic salts.

The case of *Mucor ramannianus* stands out as a glaring exception in the behavior of the other five fungi used in auxithal production. It is a uniformly poor source of thiamin and pyrimidine, the former being present in the hyphae in extremely minute amounts as shown by the behavior of *Phytophthora erythroseptica* and *Thielaviopsis basicola,* two organisms that react to mere traces (0.1 microgram per liter) of thiamin. The pyrimidine content also is very low in the mycelium, and extremely so in the medium and only the more sensitive pyrimidine-requiring organisms are capable of making a fair growth on its extracts. Both the mycelium and the medium of *Mucor ramannianus* contain thiazole, but not as much as those of the other organisms.

The inability of *Mucor ramannianus* to produce appreciable amounts of auxithals led the writers to believe that probably 1/20th p.p.m. thiazole used to produce the mycelium was not sufficient; or perhaps the fungus would do better if the pyrimidine were furnished extraneously; or if some thiamin were added to the thiazole. Consequently, the following experiments were made: a series of solutions was prepared by adding to the basic solution:

a. 1 p.p.m. thiazole
b. 1/20th p.p.m. thiazole and 1/20th p.p.m pyrimidine
c. 1/20th p.p.m. thiazole and 1/20th p.p.m. thiamin.
d. 1/20th p.p.m. thiamin

The foregoing solutions were placed in a series of Fernbach flasks (500 ml. per flask), sterilized, inoculated, incubated for 14 days and then the mycelium and the medium were treated as before; the extracts were added to the basic medium, solidified with agar, tubed, sterilized, inoculated, and incubated. Table IV shows the results.

Increasing the thiazole from 1/20 p.p.m. to 1 p.p.m. failed to bring about a significant increase in the auxithal production by *Mucor remannianus.* It appears that in the absence of an external supply of pyrimidine or thiamin the fungus synthesizes enough pyrimidine and thiamin for its immediate use, with very little left over. When, however, pyrimidine is added to the thiazole, the picture changes; the fungus synthesizes more thiamin than it needs, stores the bulk of it in the mycelium, and lets a trace of it diffuse into the medium. In addition to this, the pyrimidine content of both the mycelial

TABLE IV

THE EFFECT OF ADDING THIAMIN AND PYRIMIDINE TO THIAZOLE IN THE CULTURE MEDIUM UPON AUXITHAL PRODUCTION BY *Mucor ramannianus*

ORGANISMS	1 P.P.M. THIAZOLE		1/20 P.P.M. THIAZOLE 1/20 P.P.M. PYRIMIDINE		1/20 P.P.M. THIAZOLE 1/20 P.P.M. THIAMIN		1/20 P.P.M. THIAMIN	
	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT	HYPHAL EXTRACT	MEDIUM EXTRACT
Blakesleea trispora	VIII	VI	X	VI	X	X	VIII	VI
Chaetocladium brefeldii	II	I	V	II	V	I	IV	I
Collybia tuberosa	II	I	VII	V	VII	II	VII	II
Cyathus striatus	IV	II	V	V	IV	IV	IV	IV
Deconica inquilina	II	I	V	III	IV	III	IV	III
Gloeocystidium roseo-cremeum	II	I	X	II	X	I	X	I
Lentinus tigrinus	X	II	X	X	X	X	X	X
Lenzites saepiaria	I	I	X	III	VIII	III	VIII	III
Lenzites betulina	IV	II	X	X	VIII	VII	VIII	V
Mucor ramannianus	X	X	X	X	X	X	X	V
Phycomyces blakesleeanus	III	I	X	VII	X	V	X	I
Phytophthora erythroseptica	II	I	X	III	X	III	X	II
Phytophthora fagopyri	V	I	X	X	X	V	X	V
Pilaira moreaui	IV	II	VIII	III	VIII	III	III	III
Pythiomorpha gonapodioides	V	I	X	V	X	V	VIII	V
Pythiomorpha oryzae	I	I	VIII	I	VIII	I	VIII	I
Pythium ascophallon	I	I	X	II	X	I	X	I
Pythium oligandrum	I	I	VII	IV	VII	I	VII	II
Pythium polymastum	I	0	VI	I	VII	II	VII	II
Thielaviopsis basicola	V	I	X	V	VII	III	VI	III
Typhula variabilis	VIII	I	X	VII	X	X	X	VIII

extract and the extract of the medium is increased, and a richer growth of all organisms is the result. As seen in the last two columns of the foregoing table, 1/20 p.p.m. thiamin alone is not as favorable as a mixture of 1/20 p.p.m. each of the two moieties because, perhaps, it contains proportionately less pyrimidine and thiazole.

The increase of auxithals paralleled a very rapid acceleration of growth when pyrimidine or thiamin was added to the thiazole. A more rapid growth, however, or a larger yield of mycelium does not necessarily mean a larger percentage of auxithals per given unit of mycelium. This was demonstrated by the following experiment: 0.05 per cent. of agar-agar was added to the basic medium containing 1/20 p.p.m. thiazole; this solution was placed in Fernbach flasks, sterilized, inoculated with *Mucor ramannianus* and incubated for 14 days. An increase of mycelium of about 50 per cent. followed the addition of agar-agar to the nutrient solution. This growth-accelerating property of agar is caused by the minor elements present in it. When the mycelium and the medium were treated as before, however, and tested, they failed to induce in the test organisms a better growth than did the mycelium formed in the agar-free solution.

The biotin content of the mycelium and the medium of variously treated cultures of *Mucor ramannianus* failed to show any significant increase. This would probably indicate that in so far as this organism is concerned, thiamin or its moieties do not exert a direct control on the biotin synthesis.

Summary

Six organisms were tested for their ability to synthesize biotin, thiamin, and the two moieties of thiamin. Two of these fungi, *Rhizopus suinus* and *Fusarium niveum*, are capable of synthesizing biotin and thiamin from pure dextrose, amino acids, and inorganic salts. *Phytophthora erythroseptica* is unable to grow without an external supply of thiamin but can synthesize biotin; *Phycomyces blakesleeanus*, if furnished with pyrimidine and thiazole will link them together and produce thiamin; *Pythiomorpha gonapodioides*, when supplied with pyrimidine, will elaborate its own thiazole, link it with pyrimidine, and form thiamin; *Mucor ramannianus* can synthesize thiamin if the medium contains thiazole, the fungus being able to produce its own pyrimidine. All three organisms, Phycomyces, Pythiomorpha, and Mucor can synthesize biotin, as shown by the growth of biotin-requiring fungi in the presence of the extract of the mycelium or the medium of these fungi.

Mucor ramannianus produces less biotin and thiamin than the other five fungi. When both thiazole and pyrimidine are added to its medium instead of thiazole alone, however, the resulting mycelium supports good growth in organisms that must have either thiamin or the moieties.

The bulk of thiamin is stored in the mycelium and only traces of it pass into the medium; appreciable quantities of pyrimidine and thiazole, how-

ever, diffuse into the nutrient solution. The same applies to biotin except that comparatively larger amounts of biotin find their way into the medium.

WEST VIRGINIA UNIVERSITY
MORGANTOWN, WEST VIRGINIA

LITERATURE CITED

1. BONNER, JAMES, and ERICKSON, JAMES. The Phycomyces assay for thiamin (vitamin B_1): the method and its chemical specificity. Amer. Jour. Bot. **25**: 685–692. 1938.
2. FRIES, NILS. Über die Bedeutung von Wuchsstoffen für des Wachstum verschiedener Pilze. Symbolae Botanicae Upsalienses III: 2. 1938.
3. KÖGL, F., and TÖNNIS, B. Über das Bios-Problem. Darstellung von krystallisierten Biotin aus Eigelb. Zeitschr. physiol. Chem. **242**: 43–73. 1936.
4. ————, and FRIES, N. Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Zeitschr. physiol. Chem. **249**: 93–110. 1937.
5. LEONIAN, L. H., and LILLY, VIRGIL G. Studies on the nutrition of fungi. I. Thiamin, its constituents, and the source of nitrogen. Phytopath. **28**: 531–548. 1938.
6. ROBBINS, WILLIAM J., and KAVANAGH, F. Intermediates of vitamin B_1 and the growth of Torula. Plant Physiol. **13**: 611–619. 1938.
7. SCHLENKER, GERHARD. Die Wuchsstoffe der Pflanzen: Ein Querschnitt durch die Wuchshormonforschung. J. F. Lehmanns Verlag, München, Berlin. 1937.

MEASUREMENT OF THE RESPIRATORY QUOTIENT OF PLANT TISSUES IN A CONSTANT GASEOUS ENVIRONMENT

ALBERT ULRICH

(WITH THREE FIGURES)

Introduction

In the course of plant physiological investigations it is often necessary to determine the respiratory activity of living tissues. The rate of carbon dioxide evolution has been used frequently as an index of respiration since it is relatively easy to measure. Under some conditions, however, the carbon dioxide released does not reflect certain biochemical changes associated with respiration which may be more closely correlated with oxygen absorption. If both gases are measured simultaneously, and the respiratory quotient (CO_2/O_2) calculated, a better interpretation of attendant phenomena may be given than if an analysis is made of only one gaseous component of the system. For example, erroneous conclusions may be drawn concerning the influence of low oxygen tensions, and the effects of various absorbed salts on the formation of organic acids in excised barley roots when CO_2 measurements alone are used as a basis for measuring the respiratory processes.

Many methods for determining the respiratory quotients of living tissues have been proposed according to which samples of gas were withdrawn from an enclosed chamber and then subsequently analyzed for carbon dioxide and oxygen. Objections to these methods were based on changes in composition of the gaseous environment during the experimental period and in the necessity of great accuracy in the analytical estimations. Major improvements were developed by FERNANDEZ (**2**), who provided a closed system with a Bunsen valve pump for circulating the gases through alkali towers to absorb the carbon dioxide, and an oxygen generator controlled by an operator to replace electrolytically an oxygen deficit indicated by a manometer. His apparatus was adopted in a slightly modified manner by WETZEL (**7**) and more recently in revised form by BENNET-CLARK (**1**). The latter investigator introduced a non-pulsating pump and the automatic addition of oxygen.

Description of apparatus

The apparatus as originally designed by these workers has been simplified and changed in certain respects (fig. 1). The pump used (fig. 2) was a modification of that originally designed by BENNET-CLARK (**1**). Reiset absorption towers (D, fig. 1) as modified by STEWARD (**4**) were substituted for Pettenkofer tubes to absorb the carbon dioxide evolved from the plant tissues. The quantity of oxygen absorbed by the plant tissues was measured

by weighing the copper (or silver[1]) deposited on the cathode of the coulometer, O, connected in series with an oxygen generator, L (symbols refer to fig. 1). The volume, and therefore the composition of the circulating gas, were automatically maintained by means of a mercury manometer, K, which operated the entire electrical circuit. The fluctuations of the manometer resulting from the surging of the alkali in the absorption tower and from the slight pulsations of the pump were eliminated by connecting one arm of the manometer to the plant chamber, G. Uniform aeration of the submerged plant tissue was provided by matched sintered glass aerators F (3).

Construction and operation of pump

Details, drawn to scale, for the construction of the pump are shown in figure 2. Figure 2A is a longitudinal section and 2B and 2C are cross sections at K and M respectively. The pump shaft C is driven by a one-quarter horse power motor through the worm A, and gear B, at an approximate speed of 120 r.p.m. The $\frac{3}{4}$ inch steel shaft C, resting upon the ball bearing P, is held in a vertical position by brass bearings D and Q. D and Q are threaded to the brass cylinder F ($4\frac{3}{4}$ inches in diameter and $14\frac{3}{8}$ inches in length), the latter enclosing the three rollers K. The duralumin rollers ($\frac{3}{4} \times 2\frac{1}{4}$ inches) are hollow cylinders sliding on the rotating monel metal axels J ($\frac{3}{8} \times 4\frac{3}{4}$ inches), the rollers being held in place by the ridges L, and by the slots in M (fig. 2C). M is a brass spool held firmly in place by the pin through the shaft C. N and O are essentially truncated cones having a height of $1\frac{1}{4}$ inches and diameters of $3\frac{7}{8}$ and $1\frac{13}{16}$ inches. O is soldered to the shaft C, while N is movable but held in place by the spring G, and collar H. The collar V prevents the upward movement of the shaft when the pump is in motion. The slit I, in the cylinder F, facilitates the adjustment of the spring tension by changing the position of H to accommodate different sizes of rubber tubing S. Ordinary flexible black rubber tubing having a $\frac{1}{4}$-inch bore and a $\frac{1}{16}$-inch wall gave an air flow of 400 ml./min. R is a brass cylinder threaded to fit Q, thus forming a basin which holds the glycerin U, necessary to lubricate the rollers and rubber tubing.

To assemble the pump, the rubber tubing in the form of a loop is passed through T (a $\frac{1}{2} \times 1\frac{5}{8}$-inch hole in the brass tubing F) and then stretched tightly around the rollers of the shaft assembly (without F, D, and Q, and the gears) (fig. 2B). The cylinder F is slipped over the rubber tubing and set up as shown in fig. 2A. The flange on D serves to suspend the lower end of the pump R in the water bath after the cylinder F has been placed into a semi-circular notch cut into the board E. In order to replace the rubber tubing it is not necessary to take the pump apart, but to tie the ends of the

[1] In the present investigation it was found that Cu could be satisfactorily substituted for Ag in the coulometer. 5.68 mg. Cu = 1 ml. O_2 at standard conditions.

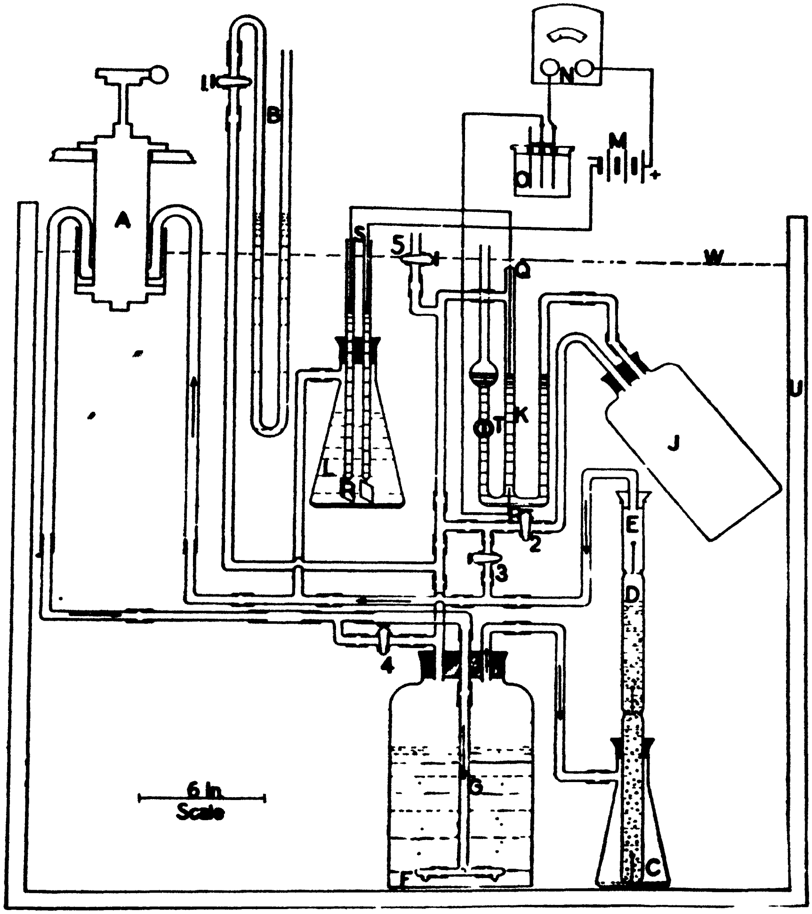


FIG. 1. Apparatus for determining the respiratory quotient of living tissues. The symbols designate the following:

A. Pump. See figure 2 and description in text.
B. Water manometer, 7-mm. glass tubing.
C.[3] 250-ml. Pyrex suction flask containing 100 ml. of NaOH.
D. Modified Reiset absorption tower. Pyrex glass tubing 2.5 cm. (outside diameter) × 43 cm.
E. Silver disk with perforations 0.022 inch in diameter.
F. Four matched sintered glass aerators.
G.[3] Four-liter wide-mouth jar fitted with rubber stopper.
J. Air reservoir, volume approximately equal to air space of circulating system.
K. Hg manometer constructed from 7-mm. glass tubing and filled with pure Hg.
L. O_2 generator, consisting of a 500-ml. suction flask containing $CuSO_4$ solution (150 gm. $CuSO_4 \cdot 5\ H_2O$ plus 1000 gm. H_2O) and solid $Cu(OH)_2$.

[3] The connections between G and C should be at an absolute minimum in order to avoid the escape of carbon dioxide through the rubber tubing.

old and new tubing together by a flexible wire, and then pull the new tubing over the rollers.

Operation of apparatus

After the apparatus has been assembled as indicated in figure 1 and the temperature of the system equalized to that of the water bath, short circuit the coulometer O to prevent the deposition of Cu on the weighed cathode, open stopcocks 1 to 5 to permit the pressure throughout the entire apparatus to become equal to the pressure of the atmosphere, and then adjust the mercury level in the arm Q of K so that it is just "off" as indicated by the ammeter N. After the mercury manometer has been once adjusted, it may be used many times, although it should be checked both before and after each determination. Next, disconnect the electrical circuit, add the plant material to G, close the stopcocks in the order of 5, 2, 3, and 4, and finally, if there is no change in pressure in the system as shown by the water levels in B, close number 1. Reconnect the electrical circuit so that copper is deposited upon a freshly prepared copper cathode in O and start the pump A. At this point it would be well to mention that if stopcock 1 is allowed to remain open when the pump is in operation, there should be a very slight positive pressure in the system as recorded by B. This pressure causes a slight depression of the mercury manometer, but as soon as a small amount of oxygen is absorbed by the plant material, the mercury in K is drawn up to the point on Q to close the circuit, and oxygen is generated within a few seconds to restore the pressure in the system. At the end of the run, stop the motor, open the electrical circuit at O, open stopcocks 3 and 4, and then close the circuit again at O. If, as previously stated, the pressure on the

---

M. 6-volt storage battery.

N. Ammeter.

O. Coulometer (voltameter). The center cathode is of electrolytic copper,[3] 0.008 inch thick and about 20 cm.[2] in area. The anodes are of heavy pure Cu. The electrolytic solution is prepared by adding 150 gm. of $CuSO_4 \cdot 5H_2O$, 50 gm. concentrated H_2SO_4, and 50 gm. 95 per cent. alcohol to 1000 gm. of water.

P and Q. Tungsten wires. Q is sharpened to a fine point by fusing with $NaNO_2$.

R. Pt plates 2–3 cm.[2] in area.

S. 7-mm. glass tubes filled with Hg.

T. Ground glass stopcock.

U. Constant temperature bath.

W. Water line.

Numbers 1, 2, 3, 4, and 5 are ground glass stopcocks.

[3] The Cu electrodes were prepared by submerging them in a vessel containing 1:1 nitric acid. When the surfaces were clean, distilled water was added and allowed to overflow the vessel until the acid became very dilute. The plates were removed and thoroughly washed with distilled water, followed by a small amount of 95 per cent. alcohol. The electrodes were dried over a sand bath and placed in a desiccator before weighing.

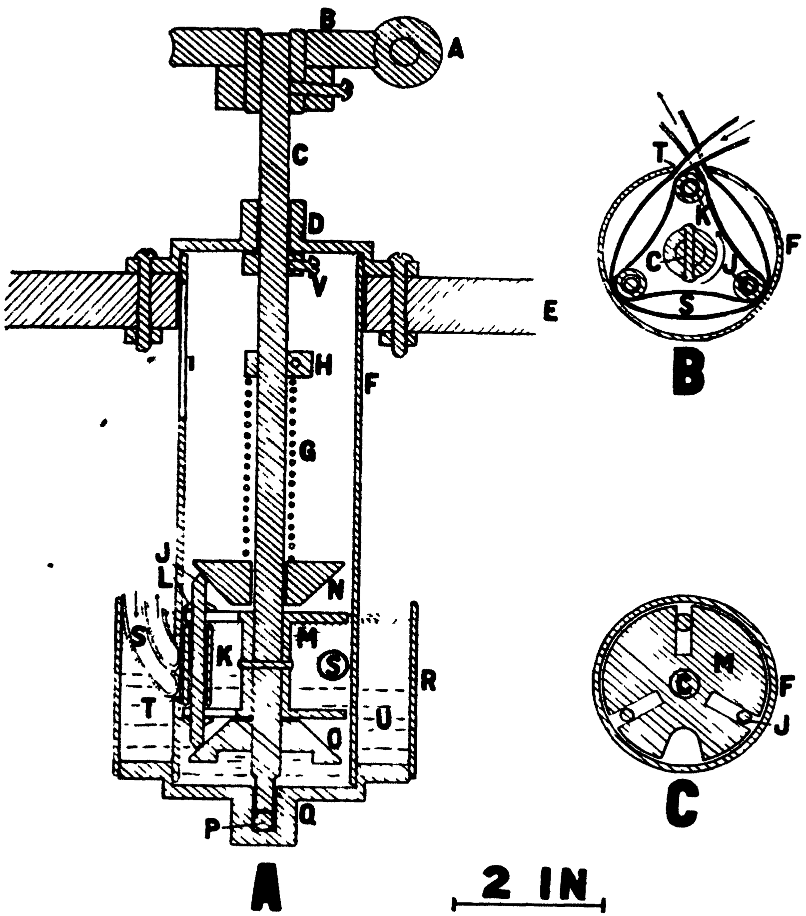


FIG. 2. Longitudinal section of pump (A) and cross sections (B) and (C) at K and M respectively. See text for explanation of symbols.

mercury manometer has been slightly positive, then a very slight reduction in the total volume of the system is necessary before oxygen can be generated to replace that absorbed by the plant material. In order to restore the original volume in the system, oxygen is generated for approximately 15 seconds until the mercury manometer is again just "off." (A negative pressure on the mercury manometer at the start cannot be readily compensated for at the end of the experiment. A negative or positive pressure can usually be obtained by changing the resistance of the sintered glass aerators, F). When the equilibrium has been restored, weigh the copper cathode, remove the tower containing alkali (usually 0.1 to 0.5 N NaOH) and determine the CO_2 evolved by WINKLER's method (6).

Oxygen tensions

The oxygen tension in the circulating system may be changed as desired by introducing a gas of known composition into the apparatus at the start of the experimental period. After placing the plant material into chamber G, a convenient procedure is to force the gaseous mixture (oxygen and nitrogen) from the storage cylinder X through the apparatus by pump A until the air has been completely displaced. The schematic drawing (fig. 3,

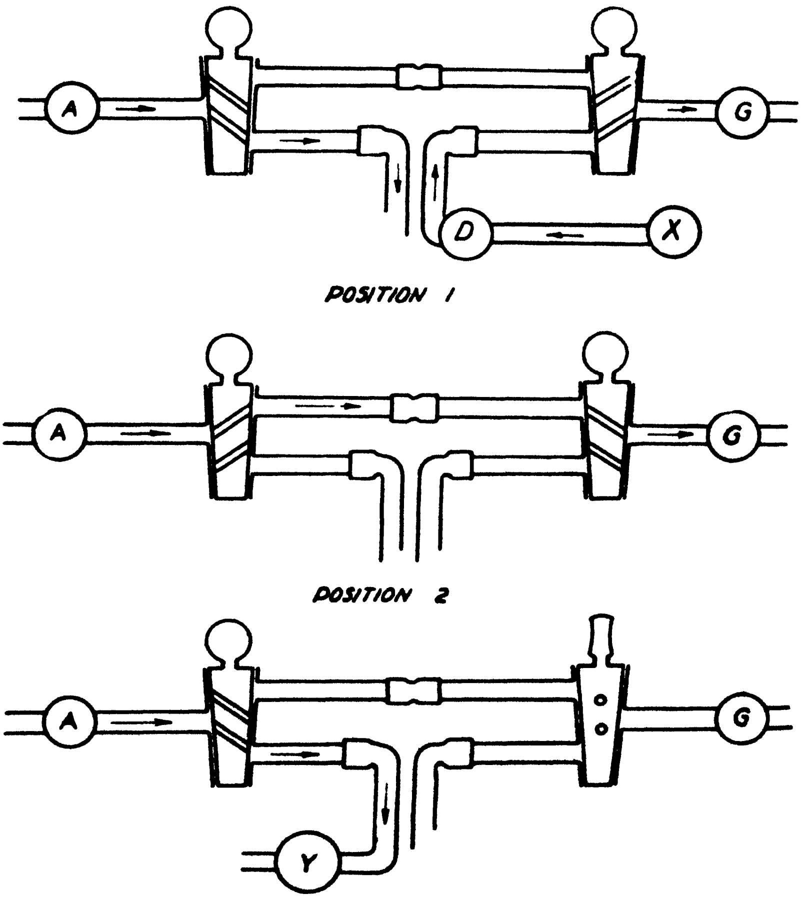


Fig. 3. Arrangement of equipment for introducing various oxygen tensions into the apparatus. A, pump; D, Reiset absorption tower; G, plant chamber; X, gas cylinder; Y, glass bulb.

position 1) indicates the arrangement of the necessary equipment. After the apparatus including the by-passes has been aerated sufficiently, the pump and gas supply are turned off, the stopcocks turned to position 2, and

thereafter the procedure is the same as outlined in the operation of the apparatus. If, at the end of the experimental period, a sample of the enclosed gas is desired for analysis, it may be withdrawn by turning the stopcocks to position 3, and then pumping the gas into a glass bulb Y, using the technique ordinarily adopted for gas analyses.

Calculations

One of the distinct advantages of the system just outlined is the ease with which the results are calculated. In those procedures which depend upon observed pressure changes in order to determine the oxygen absorbed in a closed system, numerous corrections must be made for fluctuations in atmospheric pressure while operating the apparatus. At the same time there must also be considered the usual temperature, pressure and volume relationships of gases under varying experimental conditions. These troublesome calculations are entirely eliminated when once the electro-chemical equivalence between the oxygen generated and the deposited copper is ascertained. This simplification is due to the fact that the oxygen absorbed by the plant material is returned to the closed system under exactly the same conditions as those under which it was removed and consequently the weight of the copper deposited in the coulometer is directly related to the oxygen required to restore the equilibrium. The determination of the carbon dioxide evolved offers no difficulties in this or most other systems (1 ml. of 0.1 N NaOH neutralized is equivalent to 1.113 ml. CO_2 under standard conditions).

The electrolytic reactions which occur in the oxygen generator containing a solution of copper sulphate and solid copper hydroxide are as follows:

Ionization of water:

$$H_2O \rightleftarrows H^+ + OH^-$$

In the cathode compartment:

$$2H^+ + Cu(OH)_2 \rightarrow Cu^{++} + 2\,H_2O$$

$$Cu^{++} + 2E \rightarrow Cu \text{ (deposited on cathode)}$$

In the anode compartment:

$$2\,OH^- \rightarrow H_2O + \tfrac{1}{2}\,O_2 \text{ (evolved)} + 2E$$

Normally in electrolysis the hydrogen ions which are attracted toward the cathode form hydrogen gas, but in the presence of an excess of Cu $(OH)_2$, they are neutralized by the OH ions and copper is deposited instead. Under these conditions four equivalents of copper (127.14 gm.) are deposited for each mole of oxygen generated (22,400 ml. at 760 mm. and 0° C.). Accordingly, one cubic centimeter of oxygen is equivalent to 5.68 mg. of copper.[4]

[4] In a recent communication from Dr. F. C. Steward, the formation of oxidation products other than oxygen, particularly ozone, was noted in small quantities during the

TEST RUNS

The apparatus was tested in several ways in order to determine the efficiency of the carbon dioxide absorption and the extent of the replacement of the gas by oxygen. The first series of blank determinations were designed to ascertain the recovery of carbon dioxide only. Known amounts of solid C.P. potassium bicarbonate (Merck's blue label) were introduced into a 300-ml. Erlenmeyer flask just ahead of the chamber G (fig. 1). This was followed by a small crucible containing approximately 5 ml. of 1:1 H_2SO_4. After the entire apparatus has been swept free of carbon dioxide and the absorption tower replaced with one containing fresh alkali, the flask was shaken vigorously, spilling the acid onto the potassium bicarbonate. The carbon dioxide released is recorded in table I.

TABLE I

CARBON DIOXIDE RECOVERY FROM POTASSIUM BICARBONATE

NUMBER	CO_2 AS $KHCO_3$ ADDED	CO_2 RECOVERED	RECOVERY
	ml.	*ml.*	%
21	44.8	45.7	102.0
22	44.8	44.7	99.8
31	44.8	44.8	100.0
33	44.8	44.8	100.0
23	67.2	66.1	98.4
24	67.2	66.2	98.5

Since the carbon dioxide recovery was satisfactory, a second test was undertaken in which the flask of the previous series was replaced by a glass bulb having a stopcock at each end. When this bulb was filled with carbon dioxide at the prevailing atmospheric pressure and when its temperature was that of the apparatus, it was connected into the circulating system just ahead of the respiratory chamber G. The carbon dioxide which was absorbed by the alkali scrubber was replaced by generated oxygen. The results from several determinations using the two bulb sizes are given in table II.

Typical results from excised barley roots taken from plants grown under standard conditions and subsequently subjected to a uniform treatment are given in table III. The fluctuations noted in the table were due to the variations in the root material itself and to the errors in measurement of respiration. A better criterion for evaluating the apparatus would have been through the use of absolutely uniform plant material.

generation of oxygen. Through the use of Schiff's nitrometer, the average quantity of copper deposited per ml. of oxygen (at 760 mm. and 0° C.) was found in this laboratory to be 5.75 ± 0.0071 mg. The figure 0.0071 is the standard error of the mean of 16 separate determinations. The difference of 0.07 mg. (or 1.2%) from the calculated value of 5.68 mg., while significant, is within the limits of error of the oxygen determination with the described apparatus and well within the variation of most biological material.

TABLE II

REPLACEMENT OF ABSORBED CARBON DIOXIDE BY GENERATED OXYGEN

NUMBER	SIZE OF BULB	CO_2 ABSORBED	O_2 GENERATED	CO_2/O_2
		ml.	*ml.*	
1	Small	50.80	50.14	1.013
2	Small	50.18	50.39	0.996
3	Large	67.14	63.91	1.051
4	Large	66.03	65.49	1.008
5	Large	66.56	66.14	1.006

TABLE III

RESPIRATORY ACTIVITY OF 100 GRAMS OF COMPARABLE EXCISED BARLEY ROOTS

NUMBER	CO_2 PRODUCED	O_2 ABSORBED	R.Q. CO_2/O_2
	ml.	*ml.*	
422	174.5	180.5	0.967
426	171.6	183.1	0.937
566	172.4	190.5	0.905
608	180.6	193.2	0.935
732	172.7	180.3	0.958
752	168.5	189.5	0.889

Summary

A description is given of a simplified apparatus for determining simultaneously the carbon dioxide evolved and the oxygen absorbed by plant tissues under a constant gaseous environment: (1), to permit the introduction of Reiset absorption towers for absorbing carbon dioxide; and (2), oxygen absorbed by the plant tissues was replaced electrolytically by a generator operated automatically by a mercury manometer. The quantity of current passing through the generator was measured by a coulometer in series. This method was successfully applied to a study of respiration in excised roots for the purpose of gaining further information on organic acid metabolism as related to the absorption of cations and anions (5).

Acknowledgements are made to MR. P. R. STOUT and to MR. E. J. HOFF for the technical assistance in re-designing and constructing the pump.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BENNET-CLARK, T. A. A method for automatically recording the oxygen intake of living tissues. Sci. Proc. Roy. Dubl. Soc. **20**: 281–291. 1932.
2. FERNANDES, D. S. Aerobe und anaerobe Atmung bei Keimlingen von *Pisum sativum*. Rec. trav. bot. néer. **20**: 107–138. 1923.

3. FURNSTAL, A. H., and JOHNSON, B. Preparation of sintered pyrex glass aerators for use in water culture experiments with plants. Plant Physiol. **11**: 189–194. 1936.
4. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
5. ULRICH, A. Ph.D. Thesis, University of California, 1939.
6. WINKLER, C. See TREADWELL, F. P., and HALL, W. T. Analytical chemistry. Vol. II. John Wiley and Sons. P. 485. 1924.
7. WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. IV. Zur Entstehung der Oxalsäure. Planta **4**: 476–525. 1927.

MICRO DETERMINATION OF SOME CONSTITUENTS OF PLANT ASH[1]

MONROE E. WALL

Introduction

Macro-gravimetric or volumetric procedures involved in the determination of the elements found in plant ash require a large sample and many of the operations are time consuming and tedious. Micro determinations, on the other hand, are much more rapid, require much less tissue, and are convenient to use. If very small quantities are involved, these micro methods are also more accurate; macro methods will give better results, however, if a large enough sample is obtainable.

In many investigations, comparative results are the primary objective. The ash absorption of plants grown at various potassium levels is an example. Here the use of more convenient and rapid micro methods is permissible. On the other hand, there are many instances where the amount of sample available is so small, that micro methods are not only accurate but also necessary tools.

The object of this paper is to describe certain micro methods used in determinations for constituents of plant ash. The procedures described are mostly colorimetric, involving the use of a simple, inexpensive, photoelectric colorimeter. With suitable standards, the visual colorimeter, or spectrometer, may be used in place of the photoelectric colorimeter.

Methods and results

SOLUBLE MINERALS

One-half to one gram of dry, finely pulverized plant material is placed in a 250-ml. wide mouthed flask. One hundred ml. of distilled water are added and the suspension is refluxed for one hour. The residue is filtered off by suction and washed thoroughly with hot water. A negative or faintly positive diphenylamine test for nitrates on Nessler's test for ammonia on a drop of the washings are good criteria for complete extraction of soluble minerals.

The filtrate is evaporated to approximately 50 ml. and then transferred to a 100-ml. volumetric flask and made up to volume. A few drops of chloroform and toluene are added and the flasks are kept in a cool place. Sodium, potassium, calcium, magnesium, and sulphates can be determined as in total ash analyses. Nitrates may be determined on a decolorized

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Vegetable Gardening. This investigation was conducted under a Fellowship Grant from the American Potash Institute.

aliquot (5). To determine phosphates, a 5-ml. aliquot is placed in a 50-ml. beaker, and approximately 0.1 gm. of Darco charcoal is added and stirred for one to two minutes. The material is then filtered into a 100-ml. volumetric flask and the filter washed thoroughly. The determination is then made as for total ash.

TOTAL ASH

One-half to one gram of dry, finely pulverized plant material is ashed in a muffle at 600° C. for one hour. The ash is weighed if desired, and then taken up in 50 ml. of 1 : 4 hydrochloric acid. Most of the nitrates are lost in ashing by this procedure, and hence nitrates must be determined on the aqueous extract. Plants grown on soil should have the silica removed at this point by evaporating the ash solution to dryness and heating for one hour on a water bath. The residue is moistened with 5 to 10-ml. concentrated HCl, and 25 ml. of water are added. The solution is heated for a few minutes on a warm water bath, and is then filtered through a hard filter paper and washed thoroughly. The filtrate is evaporated to approximately 25 ml. to drive off most of the HCl and is then made to volume in a 100-ml. volumetric flask (1).

This procedure is not necessary for plants grown in sand or water culture since the silica content is low. In this case the ash solution is evaporated to 25 ml. and made to volume in a 100-ml. volumetric flask.

COLORIMETRIC DETERMINATION OF PHOSPHATES

PRINCIPLE.—When phosphates react with the molybdate ion a phosphomolybdenum complex is formed. This compound when reduced will give a blue color (3). Arsenates and silicates will interfere. Arsenates are not found to any appreciable extent in plant ash. Silicates are also low in plants grown in sand and water culture. Silicates may be removed if necessary by appropriate technique (1).

REAGENTS.—Five per cent. ammonium molybdate in $2N-H_2SO_4$, 1 per cent. hydroquinone in $2N-H_2SO_4$, and 16.5 per cent. sodium bisulphite.

STANDARD.—KH_2PO_4—0.4380 gm. in one liter; 1 ml. = 0.1 mg. phosphorus.

PROCEDURE.—A 5-ml. aliquot of ash solution is pipetted into a 100-ml. volumetric flask. Four ml. of ammonium molybdate, 2 ml. of hydroquinone, and 4 ml. of sodium bisulphite solution are added. The flask is shaken, and the solution made to volume, stoppered, and set aside for one-half hour. It is convenient to run six samples in duplicate, spacing each set of duplicates 5 minutes apart. At the end of one-half hour, the colorimeter readings are taken. The standard curve is prepared in the same manner, except that aliquots can be any volume desired.

RESULTS ON KNOWN PHOSPHATE SAMPLES.—In this and other discussions of the accuracy of the methods presented, it will be assumed that the

knowns are present in the same amount of total sample as the unknowns. For instance, if 0.500 gm. plant material is ashed, dissolved, and made up to a volume of 100 ml., then a 5-ml. aliquot (the quantity used in the phosphate determination) would correspond to 25 mg. The percentage of known phosphate is calculated using this quantity as a basis of estimation.

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.480	0.492	+ 0.012	2.5
0.560	0.584	+ 0.024	4.3
1.000	0.980	− 0.020	2.0
1.120	1.080	− 0.040	3.6
1.440	1.460	+ 0.020	1.4
1.680	1.700	+ 0.020	1.2
2.200	2.128	− 0.072	3.3
3.000	3.000	0.000	0.0
3.520	3.540	+ 0.020	0.6
		Average	2.0

THE DETERMINATION OF MAGNESIUM

PRINCIPLE.—Magnesium is separated from calcium as mentioned later. (*Cf.* p. 544.) The magnesium is precipitated as $MgNH_4PO_4$ (**10**), and the magnesium estimated from the phosphate content.

REAGENTS.—Two per cent. $NH_4H_2PO_4$, concentrated NH_4OH, and four per cent. NH_4OH.

STANDARD.—$Mg(NO_3)_2$ 6 H_2O—10.538 gm. in one liter; 1 ml. = 1 mg. magnesium. Dilute if necessary.

PROCEDURE.—The supernatant washings from the calcium determination are decanted into a 50-ml. beaker, and evaporated until salts begin to crystallize out (usually 2 to 3 ml. volume). The solution is then quantitatively transferred to a 15-ml. centrifuge tube, using a small funnel. The beaker is washed three times, using not more than 2 ml. of water each time. The centrifuge tube is then placed in a boiling water bath and 2 ml. $NH_4H_2PO_4$ are added. Two ml. concentrated NH_4OH are added and stirred with a small rod. The rod is rinsed and the centrifuge tube is corked and allowed to stand over night. The precipitate of $MgNH_4PO_4$ is centrifuged and washed as discussed under calcium, the washings being discarded. The precipitate is dissolved in 2 ml. N H_2SO_4 and the resultant solution washed into a 100-ml. volumetric flask. Proceed as outlined under phosphates. The concentration of magnesium can be found by multiplying the mg. of phosphorous by 0.784.

The standard curve should preferably be made from a magnesium standard which is converted to $MgNH_4PO_4$. Good results were secured

using the phosphate standard if a blank of 0.2 mg. was subtracted from the final result. This blank was quite constant, whereas a series of magnesium knowns was consistently high by this value.

The following are results for magnesium in a known calcium-magnesium mixture:

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.250	0.320	+ 0.070	28.0
0.500	0.540	+ 0.040	8.0
0.750	0.680	− 0.070	9.0
1.000	1.010	+ 0.010	1.0
1.250	1.240	− 0.010	0.8
1.500	1.470	− 0.030	2.0

Pure magnesium samples in the range corresponding to the first three results were accurate to 2–4 per cent. The difficulty seems to be in separating the small amount of magnesium from calcium. Hence the sample taken should have at least 1 per cent. magnesium; *i.e.*, 0.1 mg. magnesium in a 2-ml. aliquot of plant ash equivalent to 10 mg. of original sample.

DETERMINATION OF POTASSIUM

PRINCIPLE.—Potassium is precipitated as the sodium potassium cobaltinitrite. The precipitate is decomposed and the nitrite determined by the sulphanilic acid-naphthylamine reaction. The composition of the precipitate varies according to the conditions, but is constant if the conditions are constant (**11**). Ammonium, but not sodium will interfere.

REAGENTS.—Thirty per cent. sodium cobaltinitrite, 10 per cent sodium chloride, 0.1 N NaOH (approx.)

Naphthylamine-sulphanilic acid solution:

7.5 gm. sulphanilic acid in 750 ml. of 10 per cent. acetic acid mixed with 7.5 gm. naphthylamine in 750 ml. of 10 per cent. acetic acid. The naphthylamine is boiled in 200 ml. of water and then 10 per cent. acetic acid is added to make 750 ml. The reagent is kept in a dark bottle well stoppered. It should be only slightly pink. If necessary the solution can be decolorized by adding activated carbon and zinc dust, heating, and filtering.

STANDARD.—KCl: 0.382 gm. per liter, 1 ml. = 0.2 mg. potassium.

PROCEDURE.—A 5-ml. aliquot of plant ash is pipetted into a 50-ml. beaker and evaporated to dryness on the steam bath. If a plant extract is used, the aliquot must be made alkaline with NaOH to drive off any ammonia present. The material is then acidified with HCl and evaporated as above.

One ml. H_2O and 2 ml. sodium cobaltinitrite reagent are added to the residue; the beaker is placed in the ice-box overnight. The precipitate is filtered through a Gooch crucible with a thin asbestos mat. The beaker and crucible are then washed with ice-cold 10 per cent. NaCl solution until the washings are colorless. The mat is picked out with a pointed rod and transferred to a 150-ml. beaker. Twenty ml. 0.1 N NaOH are added, washing the Gooch with the NaOH as it is added, the suspension is then brought to boiling. At this point the beaker should be rotated to bring all the asbestos into a uniform suspension. The decomposed cobaltinitrite precipitate is then filtered through a Gooch using a suction test-tube to catch the filtrate. The Gooch is washed well with hot water. The filtrate should be colorless or only slightly yellow.

ALTERNATIVE PROCEDURE.—One ml. plant ash is pipetted into a centrifuge tube, neutralized with NaOH and made slightly acid with HCl. Two ml. of sodium cobaltinitrite reagent are added and permitted to stand overnight in the ice-box. The precipitate is centrifuged 15 minutes at 2000–2500 r.p.m. and the supernatant liquid decanted. It is washed with 3-ml. portions of ice-cold 10 per cent. NaCl until washings are colorless, centrifuged and decanted each time as above. The precipitate is decomposed with 0.1 N NaOH, and need not be filtered.

This procedure is more accurate than the previous procedure, but the high speed and length of centrifuging make it inconvenient when many samples are to be determined.

The solution containing the decomposed cobaltinitrite precipitate is transferred to a 100-ml. volumetric flask and made to volume. A 10-ml. aliquot which should not contain more than 0.04 mg. potassium is pipetted into another 100-ml volumetric flask, and 5 ml. naphthylamine-sulphanilic acid solution is added. The solution is made to volume with 10 per cent. acetic acid and allowed to stand 5 minutes before the microammeter reading is determined.

PREPARATION OF THE STANDARD CURVE.—One and one-half grams of $NaNO_2$ are dissolved and made up to 1 liter. Twenty ml. of this solution are made to 1 liter and used to prepare the standard curve. Aliquots are transferred to 100-ml. volumetric flasks, and the color is produced and determined as above. A known potassium solution is treated in the same manner as the unknowns, and the colorimeter reading determined. Hence the nitrite curve is standardized in terms of the nitrite reading of a known potassium solution.

REMARKS.—The necessity of greatly diluting the filtrate from the decomposed cobaltinitrite precipitate results in a large dilution error. If the filtrate is not diluted, precipitation of the diazo complex occurs. In addition the pink color produced in the final estimation does not give a favorable

RESULTS OF SOME KNOWN POTASSIUM DETERMINATIONS

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
mg.	*mg.*	*mg.*	%
0.00200	0.00192	− 0.00008	4.0
0.00400	0.00384	− 0.00016	4.0
0.00600	0.00672	+ 0.00072	12.0
0.00800	0.00768	− 0.00032	4.0
0.01000	0.01090	+ 0.00090	9.0
0.01200	0.01184	− 0.00016	1.4
0.02000	0.02240	+ 0.00240	12.0
0.03200	0.03360	+ 0.00160	5.0
0.04000	0.04060	+ 0.00060	1.5
			Average 5.9

curve and a small error in reading may result in a large final error. When small quantities of potassium are to be determined, however, the method is more accurate than the $KMnO_4$ titration procedure.

DETERMINATION OF SODIUM

PRINCIPLE.—Sodium is precipitated as the uranyl zinc acetate $3UO_2(C_2H_3O_2)_2\ Zn(C_2H_3O_2)_2\ NaC_2H_3O_2\ 9H_2O$. The uranyl radical is then converted into uranyl potassium ferrocyanide, $UO_2K_2Fe(CN)_6$ (**2**). Phosphate interferes and must be removed. Calcium, magnesium, strontium, barium, iron, and potassium do not interfere.

Phosphates have been removed by hydrated lime or zinc acetate, but these methods were not so satisfactory as precipitation of the phosphate as $MgNH_4PO_4$.

REAGENTS.—Zinc uranyl acetate (**9**), 10 gm. uranyl acetate in 50 ml. boiling water containing 2 ml. glacial acetic acid, and 30 gm. zinc acetate in 50 ml. boiling water containing 1 ml. glacial acetic acid.

Mix the boiling solutions and heat again to boiling and let stand overnight. Filter and mix the filtrate with an equal volume of absolute alcohol. Let stand forty-eight hours at 0° C. and filter at 0°. The reagent is stable at room temperature.

$Mg(NO_3)_2$ – 13 gm. per 1 liter
Concentrated NH_4OH
95 per cent. alcohol
20 per cent. potassium ferrocyanide

STANDARD.—0.1271 gm. NaCl in 1 liter; 1 ml. = 0.05 mg. sodium.

PROCEDURE.—Pipette 2 ml. of ash solution into a 15-ml. centrifuge tube. Add 2 ml. $Mg(NO_3)_2$ solution and place in a water bath and heat to 100° C.

and then add 2 ml. concentrated NH_4OH, cork the tube, and let stand overnight. Centrifuge at 1200 r.p.m. for five minutes. Pipette a 2-ml. aliquot into another centrifuge tube, taking care not to stir up the precipitate of $MgNH_4PO_4$. Heat the centrifuge tube to 100° C. in a water bath for fifteen to twenty minutes. This drives off most of the excess ammonia. Acidify with 1 or 2 drops glacial acetic acid. Add 4 ml. of zinc uranyl acetate reagent and stir until precipitation begins. Let stand one hour. Centrifuge at 1200 r.p.m. for five minutes, decant, and drain on filter paper, wiping the mouth of the centrifuge tube. Wash twice with 5 ml. of 95 per cent. alcohol, centrifuging and draining as before. Dissolve the precipitate in water and transfer to a 100-ml. volumetric flask. Add four drops acetic acid and 2 ml. potassium ferrocyanide reagent. Make to volume and let stand 3 minutes. Take the reading at once.

When the alcoholic uranyl zinc acetate is added to the unknown, the alcohol may precipitate some ammonium and magnesium salts. These do not affect the determination, and are easily soluble in water.

REULTS ON KNOWN SODIUM DETERMINATIONS

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.300	0.300	0.000	0.0
0.400	0.410	+ 0.010	2.5
0.500	0.500	0.000	0.0
0.550	0.530	− 0.020	3.6
0.600	0.610	− 0.010	1.7
0.650	0.640	− 0.010	1.5
0.750	0.780	+ 0.030	4.0
0.900	0.860	− 0.040	4.4
1.100	1.040	− 0.060	5.5
			Average 3.3

VOLUMETRIC DETERMINATION OF CALCIUM

PRINCIPLE.—Calcium is precipitated as the oxalate under such conditions that the precipitation of magnesium is prevented. The precipitate of calcium oxalate is dissolved in H_2SO_4 and titrated with standard permanganate. The procedure is essentially that of KRAMER and TISDALL (8).

The calcium in the dissolved calcium oxalate can also be determined colorimetrically by precipitating as the phosphate, and then determining the phosphate ion colorimetrically. This procedure is more laborious.

REAGENTS.—Four per cent. ammonium oxalate, 4 per cent. ammonium hydroxide, 30 per cent. sodium hydroxide, 1:15 hydrochloric acid, 0.1 N $KMnO_4$ and 0.01 N $KMnO_4$.

STANDARD.—$Ca(NO_3)_2 \cdot 4H_2O$—5.9016 gm. per liter; 1 ml. = 1 mg. calcium. The standard may be diluted if necessary.

PROCEDURE.—Two ml. of ash solution are pipetted into a conical, 15-ml. centrifuge tube. One drop of methyl red solution is added, and 30 per cent. NaOH added drop by drop until the indicator turns yellow. (It is best to stir with a thin glass rod during this and any other operation involving the addition of reagents to this type of centrifuge tube, since diffusion is very slow. The rod can be washed with a thin stream of liquid from a hypodermic needle. This will keep the volume down.) One to fifteen HCl is added until the indicator turns barely pink. Two ml. of ammonium oxalate are added, the solution stirred, and the precipitate of calcium oxalate allowed to stand overnight. It is not essential to allow the precipitate to stand so long, but this procedure is convenient when one is working with a large number of samples.

The solution is centrifuged five minutes at 1200 r.p.m., and the supernatant liquid is decanted and saved for the magnesium determination. The precipitate is washed twice with 5 ml. of 4 per cent. NH_4OH, and is centrifuged and decanted each time as above, adding the supernatant liquid to the material for the magnesium determination. The calcium oxalate precipitate is dissolved in 2 ml. of N H_2SO_4, the centrifuge tube being heated on a boiling water bath. The resultant oxalate solution is titrated to a faint pink end point with 0.01 N $KMnO_4$, using a micro burette. In the titration it is best to keep the centrifuge tube immersed in a beaker of water at a temperature of 60 to 90° C. A blank determination should be run on the reagents and subtracted from the final result.

The 0.01 N $KMnO_4$ is made by diluting accurately standardized 0.1 N $KMnO_4$. It is used only for one day. The normality of the 0.1 N $KMnO_4$ should be checked every three to four days. The use of ceric ion with the

RESULTS OF THE DETERMINATION OF CALCIUM IN A KNOWN CALCIUM-MAGNESIUM MIXTURE

THEORETICAL	FOUND	ABSOLUTE ERROR	RELATIVE ERROR
%	%	%	%
0.250	0.220	−0.03	12.0
0.500	0.490	−0.01	2.0
0.750	0.760	+0.01	1.4
0.800	0.810	+0.01	1.3
1.000	1.030	+0.03	3.0
1.200	1.260	+0.06	5.0
1.500	1.470	−0.03	2.0
		Average	3.9

phenanthroline Fe++ indicator as a standard oxidant would obviate the necessity of frequent restandardizations since this reagent is quite stable.

Summary

1. The preparation of plant samples for mineral analyses has been described.

2. Colorimetric determinations of phosphate, magnesium, potassium, and sodium are presented together with data on the accuracy of the methods.

3. A micro volumetric determination of calcium is described and data on the accuracy of the method presented.

The author wishes to thank DR. V. A. TIEDJENS and PROFESSOR L. G. SCHERMERHORN for offering laboratory facilities and advice in carrying out this investigation.

NEW JERSEY AGRICULTURAL EXPERIMENT STATION
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

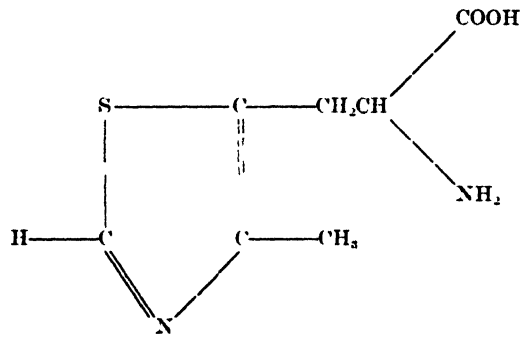
1. Assoc. Offic. Agr. Chem. Tentative methods. p. 39. 1925.
2. BARBER, H. H., and KOLTHOFF, I. M. Gravimetric determination of sodium by the uranyl zinc acetate method. Jour. Amer. Chem. Soc. **51**: 3233–3237. 1929.
3. BRIGGS, A. P. Some applications of the colorimetric phosphate method. Jour. Biol. Chem. **93**: 685–691. 1924.
4. BUTLER, A. M., and TUTHILL, E. An application of the uranyl zinc acetate method for the determination of sodium. Jour. Biol. Chem. **93**: 171–180. 1931.
5. HARPER, H. J. The accurate determination of nitrates in soils—phenoldisulfonic acid method. Ind. & Eng. Chem. **16**: 180–183. 1924.
6. JACOBS, H. R. D., and HOFFMAN, W. S. A new colorimetric method for the estimation of potassium. Jour. Biol. Chem. **93**: 685–691. 1932.
7. KAHN, B. S., and LEIBOFF, S. L. Colorimetric determination of inorganic sulfate in small amounts of urine. Jour. Biol. Chem. **80**: 623–629. 1928.
8. KRAMER, B., and TISDALL, F. F. A simple technique for the determination of calcium and magnesium in small amounts of serum. Jour. Biol. Chem. **43**: 475. 1921.
9. MCCANCE, R. A., and SHIPP, H. L. Micro determination of sodium in biological materials. Biochem. Jour. **25**: 449–456. 1931.
10. ROE, J. H., and KAHN, B. S. A colorimetric method for the determination of blood calcium. Jour. Biol. Chem. **67**: 585. 1926.
11. SNELL, F. D. Colorimetric methods of analysis. **I**: p. 433. 1936.

RESPONSE OF EXCISED TOMATO ROOTS TO β (–4–METHYLTHIAZOLYL–5) –ALANINE[1]

WILLIAM J. ROBBINS

(WITH ONE FIGURE)

The probability that thiamin (vitamin B_1) is essential for most if not all organisms makes any information on its synthesis *in vivo* of considerable importance. BUCHMAN and RICHARDSON (**2**) synthesized an amino acid, β (–4–methylthiazolyl–5)–alanine which they consider might be the precursor of the thiazole which makes an integral part of the thiamin molecule. The compound they synthesized has the following structure.



It differs from the vitamin thiazole in having a CH_2CHNH_2COOH group in place of the CH_2CH_2OH group of the latter compound. They state that the evidence accumulated to date does not permit definite conclusions regarding the biological rôle of the amino acid. BONNER and BUCHMAN (**1**), however, report pea roots able to convert the substance to the vitamin thiazole, and BUCHMAN and RICHARDSON (**2**) state that BONNER found *Phycomyces blakesleeanus* unable to bring about appreciable transformation and that KNIGHT found *Staphylococcus aureus* also unable to utilize it appreciably.

Since the strain of tomato roots cultivated in this laboratory for some years under sterile conditions is apparently able to grow indefinitely in a mineral solution containing sugar and the thiamin thiazole (**3**) it appeared worth while to determine its response to BUCHMAN's compound. A sample of the β (–4–methylthiazolyl–5)–alanine was kindly supplied by BUCHMAN.

Excised tomato roots were grown in a solution containing per liter 0.333 gm. $Ca(NO_3)_2 \cdot 4H_2O$, 0.083 gm. KH_2PO_4, 0.083 gm. KNO_3, 0.041 gm. KCl, 0.083 gm. $MgSO_4 \cdot 7H_2O$ and 0.05 p.p.m. B, 0.05 p.p.m. Mn, 0.005 p.p.m.

[1] Assistance in this work was furnished by the personnel of Works Projects Administration Official Project 65-1-97-23. W. P. no. 5.

Zn. 0.002 p.p.pm. Cu, and 0.005 p.p.m. Mo. The root fragments were grown in 50 ml. of solution in 125–ml. Erlenmeyer flasks of pyrex glass at approximately 25° C. in weak diffuse daylight. The following solutions were used: 1, the mineral solution containing 2 per cent. Pfanstiehl C. P. cane sugar; 2, the same plus per flask 10 gamma of the amino acid; 3, plus 10 gamma of the amino acid; 4, plus 10 gamma of the thiamin thiazole; 5, plus 10 gamma of the amino acid and 10 gamma of the thiamin pyrimidine; 6, plus 30 gamma of the amino acid. All solutions were sterilized at 15 pounds pressure but the amino acid was filtered sterile and added to solutions 2, 5, and 6 after the other constituents had been sterilized by heat. The amino acid in solution 3 was autoclaved along with the balance of the medium. Each of the six solutions was prepared with 5 replications and inoculated with fragments of tomato roots which had grown 24 passages during a period of 2 years in a mineral solution containing sugar and thiazole.

When examined some weeks after inoculation there was no growth in the solution containing mineral salts and cane sugar but considerable in solutions 2, 3, 4, 5, and 6. The growth in the solution 2 with the heated

TABLE I

GROWTH OF EXCISED TOMATO ROOTS THROUGH THREE SUCCESSIVE PASSAGES IN THE SOLUTIONS NOTED

Addition to mineral and sugar solution	Passage I May 4–July 13		Passage II June 28–Sept. 1		Passage III Sept. 1–Nov. 15		Range dry weights	Average dry weight per root for 3 passages
	Number of roots	Dry weight per root	Number of roots	Dry weight per root	Number of roots	Dry weight per root		
		mg.		mg.		mg.	mg.	mg.
None	2	0.6	5	1.7	5	0.2	0.1– 0.4	0.8
10 gamma (65 µ) heated amino acid	3	3.7	5	6.6	5	5.1	0.8– 9.4	5.1
10 gamma (65 µ) filtered amino acid	4	2.3	5	1.7	5	3.6	2.7– 4.3	2.5
10 gamma (68 µ) thiamin thiazole	3	9.7	5	12.9	5	7.3	5.1–10.1	.10.0
10 gamma (65 µ) filtered amino acid plus 10 gamma pyrimidine	4	14.1	5	3.7	5	6.7	0.2–12.1	8.2
30 gamma (195 µ) filtered amino acid	5	5.6	5	5.4	4	9.6	0.5–10.7	6.9

amino acid was superior to that in the solution 3 containing the amino acid filtered sterile. In neither however was growth as good as with the thiamin thiazole solution 4. Growth in solution 6 containing 30 gamma of the amino acid per flask was better than with 10 gamma.

At the end of 6 weeks subcultures were made from the best roots in each series into solutions of the same composition and prepared in the same way. Because of the small amount of growth in solution 1 no subcultures could be made and fragments of a fresh root which had grown in a thiamin solution were used. The results in the second passage were in general like those in the first passage.

At the end of 7 weeks fresh solutions of the amino acid were prepared and subcultures of the best root in each series were again made into solutions of the same composition. The results in the third passage were much like those in the first and second. The dry weights of the roots are given in table I and typical roots in each solution at the end of the third passage are shown in figure 1.

In all three passages (table I) the effect of the 10 gamma of thiamin thiazole was superior to that of the amino acid; the 10 gamma of auto-

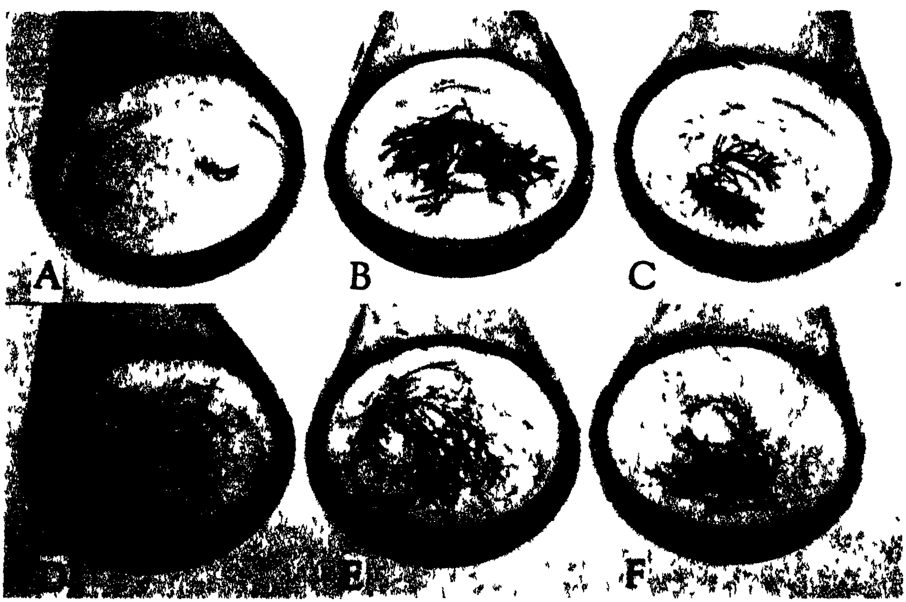


FIG. 1. Growth of tomato roots in a mineral solution containing 2 per cent. cane sugar supplemented with A, nothing; B, 10 gamma thiazolyl alanine autoclaved; C, 10 gamma thiazolyl alanine filtered sterile; D, 10 gamma thiamin thiazole; E, 10 gamma thiazolyl alanine filtered sterile + 10 gamma thiamin pyrimidine; F, 30 gamma thiazolyl alanine filtered sterile. Age, 76 days. 3rd successive passage.

claved amino acid were superior to the same amount of filtered amino acid and the 30 gamma of amino acid were superior to the 10 gamma.

It would appear from these results that the amino acid supplies the needs of the tomato root though not as satisfactorily as the thiamin thiazole. In fact, the habit of growth and general morphology of the roots in the solutions containing the amino acid were much the same as those of roots grown in the presence of the thiamin thiazole. BONNER and BUCHMAN found the amino acid effective with pea roots. On the other hand both Phycomyces and *Staphylococcus aureus* are reported to be unable to use the amino acid appreciably.

What is the explanation for the difference in the response of excised root cultures and of the microorganisms? I believe the difference is apparent only. I have found the amino acid effective with Phycomyces if enough of it is supplied, although amounts of the amino acid of the order of 1000 times those of the vitamin thiazole are required to produce the same effect.[2] This is illustrated by the experiments summarized in table II.

TABLE II

AVERAGE DRY WEIGHTS OF TRIPLICATE CULTURES OF PHYCOMYCES GROWN 6 DAYS AT 25° C. SUPPLEMENTS ADDED PER FLASK TO A SOLUTION OF SUGAR, ASPARAGINE AND MINERAL SALTS ARE GIVEN IN UNITS OF 10^{-9} GRAM-MOLE

ADDITIONS TO SOLUTION OF SUGAR, MINERAL SALTS AND ASPARAGINE	AVERAGE DRY WEIGHT	
	EXPERIMENT I	EXPERIMENT II
	mg.	*mg.*
1 μ thiamin	106.0	84.5
10 μ thiamin	117.0	......
1 μ thiazole + 1 μ pyrimidine	102.6	93.7
10 μ thiazole + 10 μ pyrimidine	122.0	......
1 μ filtered amino acid + 1 μ pyrimidine	6.3	......
10 μ filtered amino acid + 10 μ pyrimidine	10.7	......
1 μ heated amino acid + 1 μ pyrimidine	7.0	......
10 μ heated amino acid + 10 μ pyrimidine	9.8	......
100 μ filtered amino acid + 10 μ pyrimidine	......	38.8
1000 μ filtered amino acid + 10 μ pyrimidine	......	105.3
100 μ heated amino acid + 10 μ pyrimidine	......	38.8
1000 μ heated amino acid + 10 μ pyrimidine	......	106.0

In these experiments the plus strain of Phycomyces was grown in 125-ml. Erlenmeyer flasks in 25 ml. of a solution containing per liter 50 gm. dextrose, 2.0 gm. asparagine, 1.5 gm. KH_2PO_4, 0.5 gm. $MgSO_4 \cdot 7H_2O$ and the following trace elements 0.01 p.p.m. B, 0.04 p.p.m. Cu, 0.20 p.p.m. Fe, 0.02 p.p.m. Ga, 0.02 p.p.m. Mn, 0.02 p.p.m. Mo, and 0.18 p.p.m. Zn.

[2] Since the preparation of this manuscript BUCHMAN has advised me that BONNER also found the amino acid to have about 1/1000 the activity of the vitamin thiazole for Phycomyces and that KNIGHT found 10^{-6} M concentration to have no effect on Staphylococcus whereas 10^{-9} M concentration of the vitamin thiazole produced noticeable growth.

The cultures were incubated at 25° C. in faint diffuse day light. Various amounts of thiamin, mixtures of the thiamin thiazole and thiamin pyrimidine and of the amino acid and thiamin pyrimidine were added to the basic solutions. Dry weights were determined at the end of 6 days and are given in table II.

With 10^{-9} or 10^{-8} gram-mole of the amino acid growth was entirely submersed and scanty; with 10^{-7} gram-mole aerial mycelium and short sporangiophores were produced; with 10^{-6} gram-mole normal sporangia were formed and growth was as good or better than that produced by 10^{-9} gram-mole of thiamin thiazole. No difference was evident between the effects of the amino acid filtered sterile and that heated in the autoclave. It appears, therefore, that the amino acid is effective with Phycomyces if sufficient is used.

It would seem that the amino acid is much more available to the tomato root than to Phycomyces. A similar difference, however, appears to exist in the relation of these organisms to the vitamin thiazole. The lower limit for a response by Phycomyces to a thiazole pyrimidine mixture is about 10^{-11} gram-mole; a definite and measurable effect on the growth of excised tomato roots of amounts of thiazole as low as 10^{-13} gram-mole has been observed by the writer. This difference in sensitivity to thiazole may be associated with the slower growth and smaller total growth of tomato roots as compared to Phycomyces or it may be the result of some more fundamental difference.

BONNER and BUCHMAN (1) state that the amino acid is transformed *in vivo* and by the pea root into the thiamin thiazole. It is possible, however, that for Phycomyces the amino acid functions as such but is much less effective than the thiamin thiazole or that a transformation into the thiamin thiazole occurs *in vitro*. The latter possibilities appear the more probable. If Phycomyces, for example, is capable of transforming the amino acid into the thiamin thiazole one would rather anticipate a greater transformation than the growth reported here indicates. The observation that the autoclaved amino acid was more effective with tomato roots than that which was filtered sterile may have resulted from some adsorption in filtration or from a partial change of the amino acid into the thiamin thiazole *in vitro*. Failure to observe a similar effect with Phycomyces may have been because of the greater thiazole requirement of that organism. In any event, we can not yet accept the assumption that the amino acid is a normal precursor of the thiamin thiazole *in vivo*.

THE NEW YORK BOTANICAL GARDEN
and
DEPARTMENT OF BOTANY
COLUMBIA UNIVERSITY

LITERATURE CITED

1. BONNER, J., and BUCHMAN, E. R. Syntheses carried out *in vivo* by isolated pea roots: I. Proc. Nat. Acad. Sci. **24**: 431–438. 1938.
2. BUCHMAN, EDWIN R., and RICHARDSON, EDWIN M. Thiamin analogs. I. β-(4-methylthiazolyl-5)-alanine. Jour. Amer. Chem. Soc. **61**: 891–896. 1939.
3. ROBBINS, WILLIAM J., and SCHMIDT, MARY BARTLEY. Growth of excised tomato roots in a synthetic solution. Bull. Torrey Bot. Club **66**: 193–200. 1939.

SPECIFICITY OF NICOTINIC ACID AS A GROWTH FACTOR FOR ISOLATED PEA ROOTS[1]

JAMES BONNER

(WITH ONE FIGURE)

It is known that nicotinic acid is essential to the growth of numerous species of isolated roots (ADDICOTT and BONNER, 1, BONNER and DEVIRIAN, 4). The present paper is concerned with the chemical specificity of nicotinic acid as a root growth factor.

Methods

The pea roots (variety Perfection) which were used exclusively in the present experiments were cultured according to the methods outlined in earlier papers (BONNER and ADDICOTT, 3; BONNER and DEVIRIAN, 4). Four-mm. tips were cut from the roots of aseptic pea seedlings and transferred to nutrient medium containing per liter of redistilled water 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 36 mg. $MgSO_4 \cdot 7H_2O$; 81 mg. KNO_3; 65 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. ferric tartrate; and 40 gm. of sucrose. After the tips had remained in the medium for one week at 25° C., and had grown into roots several cm. long, they were subcultured by tranferring 1-cm. tips to fresh nutrient solution. This process of subculture was repeated weekly. Vitamin B_1, which is essential to the continued growth of isolated pea roots, was added at the rate of 0.1 mg. per liter to all of the nutrient solution for transfers later than the first. Nicotinic acid, or related substance to be tested for ability to replace nicotinic acid, was added at the rate of 0.5 mg. per liter, since this concentration has been found to be approximately optimal for pea roots (ADDICOTT and DEVIRIAN, 2).

A rigid selection for uniformity in root growth rate was carried out at the end of the first week of culture in order to assure the greatest possible uniformity in the roots actually used in each experiment. In a single experiment 400–500 roots were cultured and 20–50 roots were used for testing the activity of each substance reported below. In each experiment the roots were maintained through 5 or more weekly transfers.

Experimentation

The ability of decreasing concentrations of nicotinic acid to support the continued growth of isolated pea roots is shown in figure 1. Although 0.5 γ nicotinic acid per liter of nutrient solution supports modest growth, a ten times higher concentration is needed to support growth at a significant level. Still ten times higher concentration (50 γ per liter) supports growth

[1] Work carried out with the assistance of the Works Progress Administration, Official Project Number 65-1-07-98, Work Project Number 11534.

at a maximum level, beyond which further increases result in no further increases in growth rate. The standard concentration, 0.5 mg., of nicotinic acid used in these experiments is therefore at least 100 times as high as is needed for a detectable promotive influence on the growth of isolated pea roots. In the experiments reported later analogs of nicotinic acid were also used in a concentration of 0.5 mg. per liter. An analog inactive at this concentration may then be concluded to possess less than 1 per cent. of the activity of nicotinic acid in supporting the growth of isolated pea roots.

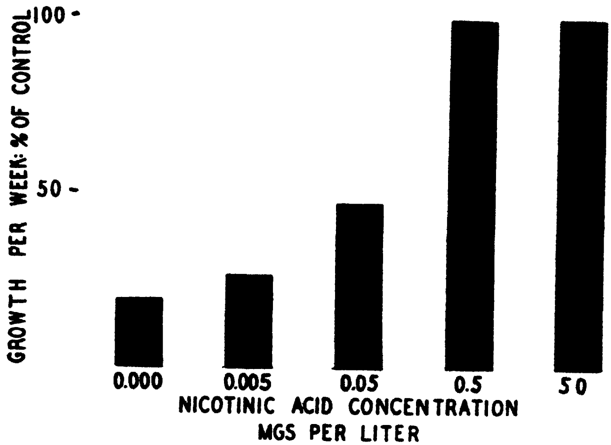


FIG. 1. Growth of isolated pea roots in medium containing adequate amounts of vitamin B_1 and varying amounts of nicotinic acid. The growth rates given are those for the fifth week of roots which had already been maintained through five weekly transfers in a medium of the composition shown.

Table I gives as an example one experiment in which methyl nicotinate and β-picoline respectively were substituted for nicotinic acid in the basic medium. In the absence of nicotinic acid growth in length decreases in each successive transfer. In medium containing nicotinic acid on the other hand, the growth rate is maintained at essentially a constant level. After 5 weekly transfers the roots which received nicotinic acid grew approximately 5 times as much per week as roots which did not receive nicotinic acid. Table I shows that the methyl ester of nicotinic acid also supports the growth of isolated pea roots and it may be concluded that methyl nicotinate is capable of replacing nicotinic acid as a growth factor for the isolated pea root. β-picoline, on the other hand, is clearly incapable of replacing nicotinic acid since roots supplied with β-picoline grew no better than the control roots which received vitamin B_1 alone.

All of the substances discussed below were tested, in experiments similar to that exemplified in table I, for ability to support the growth of pea roots through at least 5 weekly transfers. Each substance was either clearly inac-

TABLE I

ACTIVITIES OF 2 SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE CONTINUED GROWTH OF ISOLATED PEA ROOTS. 30–40 ROOTS IN EACH SERIES. ROOTS CULTIVATED FOR A PRELIMINARY WEEK IN MEDIUM CONTAINING VITAMIN B_1 AND NICOTINIC ACID

SUPPLEMENTS TO MEDIUM	GROWTH PER WEEK DURING:				
	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
Vitamin B_1, 0.1 mg./l.	63	42	35	22	16
" + nicotinic acid, 0.5 mg./l.	79	78	74	86	80
" + methyl nicotinate, 0.5 mg./l.	85	78	81	96	78
" + β-picoline, 0.5 mg./l.	58	41	31	22	16

tive and supported growth at no higher level than nicotinic acid-free medium or it was completely active and supported growth essentially as well as nicotinic acid itself. Table II gives the activities in supporting isolated pea root growth of 23 substances related to nicotinic acid. Nicotinamide, esters of nicotinic acid, and nicotinuric acid were the only compounds among the substances tested which were active in supporting the growth of isolated pea roots. These active substances are all ones which yield nicotinic acid on simple hydrolysis. It must be concluded therefore that of the substances tested, only those which readily yield nicotinic acid by hydrolysis *in vivo* are capable of replacing the latter substance in the nutrition of isolated pea roots.

TABLE II

ACTIVITIES OF SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE GROWTH OF ISOLATED PEA ROOTS. + = ACTIVE. 0 = INACTIVE. DATA OBTAINED BY EXPERIMENTS OF TYPE SHOWN IN TABLE I

1. Nicotinic acid	+	10. Picolinic acid[1]	0
2. Nicotinamide	+	11. Quinolinic acid[1]	0
3. Coramine (N-diethyl amide of 1)[1]	0	12. Dinicotinic acid[1]	0
4. Methiodide of 2[1]	0	13. Nicotinuric acid[1]	+
5. Methyl nicotinate[1]	+	14. Ethyl nicotinoylacetate[1]	0
6. Ethyl nicotinate[1]	+	15. Arecoline[1]	0
7. Propyl nicotinate[1]	+	16. β amino pyridine[1]	0
8. Butyl nicotinate[1]	+	17. Nicotino-3-nitrile[1]	0
9. Isonicotinic acid[1]	0	18. β-picoline[1]	0
		19. Thiazole-5-carboxylic acid[2]	0
		20. Amide of 19[2]	0
		21. Benzoic acid	0
		22. Pyrazine, 3, carboxylic acid[2]	0
		23. Pyrazine, 2–3, dicarboxylic acid[2]	0

[1] Obtained through the courtesy of Prof. FELIX SAUNDERS and Dr. ALBERT DORFMAN, University of Chicago.

[2] Obtained through the courtesy of Dr. FRANZ C. SCHMOLKES, Research Division, Wallace & Tiernan Products, Inc., Belleville, New Jersey.

Inspection of table II shows that the steric requirements for nicotinic acid activity are strict. Thus isonicotinic acid and picolinic acid are completely inactive, although they differ from nicotinic acid only in the position of the carboxyl group relative to the heterocyclic nitrogen atom. Substitution of a second carboxyl group as in dinicotinic acid and quinolinic acid also renders the molecule inactive. It is noteworthy that the N-diethyl substituted amide of nicotinic acid, coramine, is inactive, indicating that this substance is not hydrolyzed *in vivo* by the pea root.

The carboxylic acids of certain cyclic substances other than pyridine are unable to replace nicotinic acid in the nutrition of pea roots. Thus benzoic acid is inactive. Thiazole-5-carboxylic acid and its amide are both inactive even though in this substance, as in nicotinic acid, the carboxyl group is β to the cyclic N atom. Pyrazine-3-carboxylic acid is likewise inactive.

Vitamin B_6, which like nicotinic acid is a pyridine derivative, is completely incapable of replacing the latter in the nutrition of isolated pea roots. Similar results have been obtained with isolated tomato roots (**10**).

Discussion

In relation to the general question of which reactions are capable and which incapable of execution *in vivo,* it is of interest that the pea root can neither hydrolyze nicotino-3-nitrile nor oxidize the methyl group of β-picoline to form nicotinic acid.

The specificity of nicotinic acid in the nutrition of pea roots is in substantial agreement with the specificity of this substance in the nutrition of *Staphylococcus aureus* (**7, 8, 9**), dysentery bacillus (**5, 6**) and the dog (**11**), with the particular exception that β-picoline is claimed to possess curative activity for black tongue.

The present data do not permit of a decision as to whether it is nicotinic acid *per se* which is required as a growth factor by the isolated pea root, or whether it may not be nicotinamide which, as a constituent of the codehydrogenases (**12**), is the true active substance. On the assumption that nicotinamide is the substance actually utilized by the pea root, it must be concluded that the root is capable of ready amide formation *in vivo.*

Summary

The ability of 23 substances chemically related to nicotinic acid to replace the latter substance as a growth factor for isolated pea roots has been determined. Among those tested, only substances which yield nicotinic acid by simple hydrolysis were found to be active.

William G. Kerckhoff Laboratories of the Biological Sciences
California Institute of Technology
Pasadena, California

LITERATURE CITED

1. ADDICOTT, F., and BONNER, J. Nicotinic acid and the growth of isolated pea roots. Science n.s. **88**: 577–588. 1938.

2. ————, and DEVIRIAN, P. S. A second growth factor for isolated pea roots: nicotinic acid. Amer. Jour. Bot. **26**: 667–671. 1939.

3. BONNER, J., and ADDICOTT, F. Cultivation *in vitro* of excised pea roots. Bot. Gaz. **99**: 144–170. 1937.

4. ————, and ————. Growth factor requirements of four species of isolated roots. Amer. Jour. Bot. **26**: 661–665. 1939.

5. DORFMAN, A., KOSER, S. A., and SAUNDERS, F. The activity of certain nicotinic acid derivatives as growth essential for the dysentery bacillus. Jour. Amer. Chem. Soc. **60**: 2004. 1938.

6. ————, ————, REAMES, H. R., SWINGLE, K. F., and SAUNDERS, F. Nicotinamide and related compounds as essential growth substances for dysentery bacilli. Jour. Inf. Dis. **65**: 163–182. 1939.

7. KNIGHT, B. C. J. G. The nutrition of *Staphylococcus aureus;* nicotinic acid and vitamin B_1. Biochem. Jour. **31**: 731–737. 1937.

8. ————. The nutrition of *Staphylococcus aureus.* The activities of nicotinamide aneurin (vitamin B_1) and related compounds. Biochem. Jour. **31**: 966–973. 1937.

9. LANDY, M. Effect of nicotinic acid, its isomers and related compounds upon the nutrition of Staphylococcus. Proc. Soc. Exp. Biol. & Med. **38**: 504–506. 1938.

10. ROBBINS, W. J., and SCHMIDT, MARY. Further experiments on excised tomato roots. Amer. Jour. Bot., **26**: 149–159. 1939.

11. WOOLLEY, D. S., STRONG, F. M., MADDEN, R. J., and ELVEHJEM, C. A. Anti-black tongue activity of various pyridine derivatives. Jour. Biol. Chem. **124**: 715–723. 1938.

12. WARBURG, O. Chemische Konstitution von Fermenten. Ergeb. d. Enzymforsch. **7**: 210. 1938.

BRIEF PAPERS

TRANSMISSION SPECTROGRAMS OF LEAF EXTRACTS

FREDERICK F. FERGUSON, WILL S. DELOACH,
AND LEWIS W. WEBB, JR.

(WITH TWO FIGURES)

It is felt that biologists in general, and plant physiologists in particular, might be interested in the transmission spectrogram of a denatured alcoholic solution of leaf extracts, which solution is commonly used in the laboratory for demonstration purposes. The following curves were obtained by the

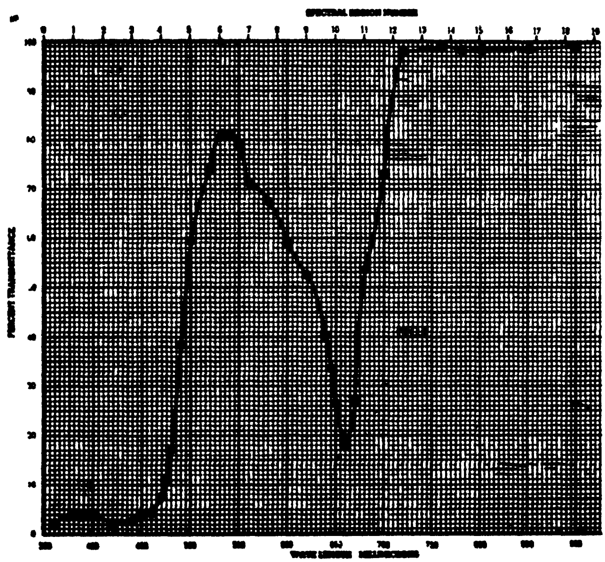


FIG. 1. Transmission spectrogram of *Poa pratensis* leaf extract using 30 mμ bands.

use of the Coleman Regional Spectrophotometer.[1] The points recorded in figure 1 represent the average transmission of bands 30 mμ in width.

The solution used was prepared in the following manner: denatured ethyl alcohol (100 ml.) was added to selected fresh green leaves of *Poa pratensis* (2 gm.) and the resulting mixture was heated for 15 minutes on a water bath under a reflux condenser. The dark green alcoholic solution was decanted and diluted by the addition of 15 parts of denatured alcohol to 5 parts of the solution. This dilution was made in order to get appreciable transmission in the region from *ca.* 360 mμ to *ca.* 450 mμ.

[1] This instrument was procured for this study through the kindly offices of the Phipps and Bird Co., Richmond, Virginia.

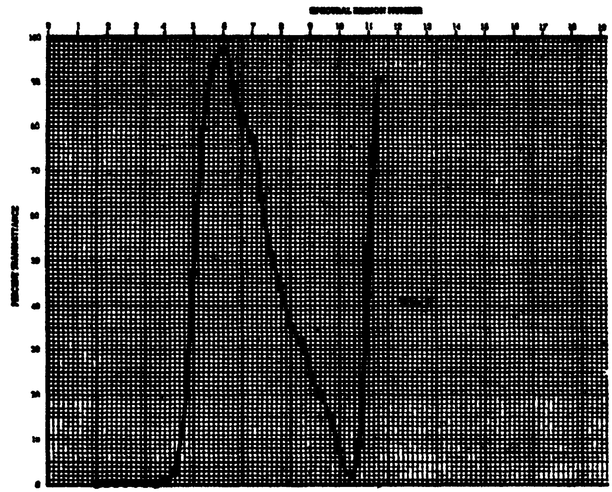


Fig. 2. Transmission spectrogram of dilute leaf extract of *Poa pratensis* using 10 mμ bands.

Figure 2 was secured by the use of bands of approximately 10 mμ in width. In this case a more dilute solution was used in order to allow the passage of more light.

College of William and Mary
Virginia Polytechnic Institute
Norfolk Division

IMPROVEMENTS IN THE SIMPLIFIED METHOD FOR OSMOTIC MEASUREMENTS

CHARLES J. LYON

The extension of URSPRUNG's simplified method (2) to the provision of data for determining osmotic quantities other than net osmotic pressure, was suggested in 1936 (1). At that time we described the immersion of cylinders of tissue, each several centimeters long, in test tubes of sugar solutions. Each cylindrical stick was measured with rule and hand lens and the length used as an index of the volume of the cylinder. Subsequent use of the method for research and classroom purposes has led to refinements in procedure.

In the place of long cylinders, it is better to cut sticks approximately square in cross section and somewhat less than 1 cm. in length. These are easily cut to the same length by use of a double-bladed knife, made by bolting two razor blades to a block of suitable width. A slice of desired thickness, preferably one-tenth the cut of the double knife, is first taken from the tuber, fruit, or other organ by a sharp, broad-bladed knife. The double knife is used to make parallel cuts through the slice; from the tissue between them, a razor blade held vertically is used to take a series of square sticks of equal length.

In place of stoppered test tubes from which the tissue must be removed for measurement, the short, square sticks should be immersed in low preparation dishes. They can then be measured accurately without danger of drying or distortion by setting each dish in turn in the field of a 10 × binocular microscope provided with a micrometer scale in one ocular. It is convenient to use 10 or 12 ml. of water, or solution, in each dish. If plant material is plentiful, several sticks of it may be placed in each dish to lower the sampling error.

It is important to hold a few control sticks of the tissue in similar dishes of paraffin oil unless the oil kills the cells rapidly. For undetermined reasons, these control sticks may shrink or expand between the start and the end of the immersion period, which need not be over 4 hours for the short sticks recommended. The final measurement of the control is the logical figure to use for the original length of stick unless the cells die and the walls contract.

If the distilled water kills the cells in the test of volume at the saturation point, no significant osmotic error is introduced by the use of good tap water. Tissues sensitive to distilled water will usually live in tap water and the volume at the saturation point is essential to the computations.

For exact work with the method, the series of solutions must be closely graded. This usually means a preliminary test to determine the approxi-

mate location of the two critical points—incipient plasmolysis and the "normal" of the tissue as it came from the plant. The point of incipient plasmolysis is always the more difficult to locate and very important for all estimates of absolute osmotic values.

It is possible to improve the accuracy of this method for determining osmotic quantities by using calculated volumes for the sticks of tissues at the three critical points. In the original description of the procedure, the length was used as an index of the volume. It is impractical to measure more than the length of each stick but if the side of the square end is made approximately one-tenth the length, it is easy to calculate a satisfactory volume by the use of a table of cubes.

DARTMOUTH COLLEGE
HANOVER, NEW HAMPSHIRE

LITERATURE CITED

1. LYON, C. J. Analysis of osmotic relations by extending the simplified method. Plant Physiol. **11**: 167–172. 1936.
2. URSPRUNG, A. Zur Kenntnis der Saugkraft. VII. Eine neue, vereinfachte Methode zur Messung der Saugkraft. Ber. d. bot. Ges. **41**: 338–343. 1923.

ISOLATION OF THE ALKALOIDS, BERBERINE AND BERBAMINE, FROM *MAHONIA SWASEYI*

GLENN A. GREATHOUSE AND NEIL E. RIGLER

In a previous communication, the senior author (**1**) recorded the isolation of berberine, $C_{20}H_{17}O_4N \cdot 2\frac{1}{2}\, H_2O$ from *Mahonia swaseyi,* Fedde (*Berberis swaseyi* Buckl.). Further studies have revealed the presence of the alkaloid berbamine in the root tissue of this species. *Mahonia swaseyi* is of limited habitat and has not been hitherto chemically examined. The resistance of this plant to *Phymatotrichum omnivorum* (Shear) Duggar and its use as an ornamental shrub in root rot areas of Texas were factors in the initiation of these chemical examinations.

The procedure for the separation of berberine has been described (**1**). Following the separation of berberine, berbamine remained in the mother liquors and was removed by addition of sodium hydroxide solution, and extraction by ether. The residue left on evaporation of the solvent was dissolved in acetic acid. From this solution, berbamine nitrate was precipitated with sodium nitrate. The alkaloid was regenerated, converted into the hydrochloride, and recrystallized from water. No other alkaloids were detected in these studies. Berberine was found (**1**) to be present in a concentration varying from 2.15 to 2.48 per cent. on the dry weight basis. Berbamine, on the other hand, was present in concentrations in the root tissue to the extent of 0.02 to 0.05 per cent.

The color reactions with H_2SO_4, and HNO_3, and the melting point of 200 to 202° C. (corrected) of the free base recrystallized from petroleum ether are those recorded in the chemical literature for berbamine.

Calculated: for $C_{18}H_{19}O_3N$,—N, 4.71%; H, 6.45%; C, 72.68%. Found:[1] N, 4.60%; H, 6.51%; C, 72.82%.

The localization of berberine and its fungicidal effectiveness on the growth of *P. omnivorum* has been reported (**1, 2**). The fungicidal power of berbamine to this fungus has been also recorded (**2**).

BUREAU OF PLANT INDUSTRY
U. S. DEPARTMENT OF AGRICULTURE, AND
TEXAS AGRICULTURAL EXPERIMENT STATION
COLLEGE STATION, TEXAS

LITERATURE CITED

1. GREATHOUSE, GLENN A., and WATKINS, G. M. Berberine as a factor in the resistance of *Mahonia trifoliolata* and *M. swaseyi* to Phymatotrichum root rot. Amer. Jour. Bot. **25**: 743–748. 1938.

[1] Average of duplicate determinations made by the Arlington Laboratories, Arlington, Virginia.

2. ————, and RIGLER, NEIL E. The chemistry of resistance of plants to Phymatotrichum root rot. V. The influence of alkaloids on the growth of fungi. Phytopath. **30**: 475–485. 1940.

CRITICISM OF A RECENT PAPER ON THE PECTIC CONTENT OF PLANT MATERIALS

Z. I. KERTESZ

A paper entitled *Pectic content of plant materials* appeared in PLANT PHYSIOLOGY a few months ago (2). ELWELL and DEHN analyzed various plant tissues and vegetables to determine the proportion of pectic constituents present. The material was first extracted with water, then twice with dilute sulphuric acid. The extracts were used for the determination of pectic materials as calcium pectate (1) and by alcohol precipitation. The results obtained on the three extracts were combined to give the total pectin content of the analyzed material. The following criticisms are offered in connection with the techniques employed by ELWELL and DEHN.

1. An alcohol precipitate from an extract from a plant tissue, or even from a fruit, may include a great many other compounds besides the pectins. Starch, dextrins, hemicelluloses, proteins, and many other plant constituents are extracted by hot water and precipitated by the addition of alcohol. Thus while the precipitates undoubtedly *included* pectins, the pectin may have constituted only a fraction of the total alcohol precipitate, designated in the article as pectin.

2. While the unspecific precipitation by alcohol includes many materials in addition to pectin, the determination of the pectins as calcium pectate, if properly carried out, is specific for the pectic materials. Consequently the percentage of alcohol precipitate obtained from crude plant extracts is always higher than the percentage of calcium pectate determined in the same extract. From the 15 materials analyzed by ELWELL and DEHN by both methods the percentage of "pectin as calcium pectate" is in nine cases higher than the percentage of total alcohol precipitate.

This discrepancy between the two methods is most apparent from the results given in table I for the fresh and stored cranberry (berry). The results, expressed by the authors on the dry matter basis, are as follows:

	PECTIN AS CALCIUM PECTATE	PECTIN AS ALCOHOL PRECIPITATE
	%	%
Cranberry, fresh	50.6	2.74
Cranberry, stored	16.53	9.77

An observation of the results makes further discussion of this point superfluous.

3. The percentage of "pectin as alcohol precipitate" (it should be "alcohol precipitate as pectin") as reported by ELWELL and DEHN is in many

cases so high that it will be obvious to all concerned with the analysis of plant materials that the figures are either incorrect or do not represent "pectin." For instance, the solids of asparagus are claimed to contain 28.54 per cent.; dandelion greens 31.42 per cent.; lettuce 24.99 per cent.; rhubarb 90.9 per cent. (!) and Swiss chard 70.80 per cent. pectin. Again no discussion of these percentages is needed to demonstrate the obvious impossibility of these results.

It appears from the foregoing that ELWELL and DEHN either overlooked an important detail in the chemical methods used or that some mistake was made in calculating the results. In either case it would be most unfortunate if the alleged pectin contents of various plant materials, as reported by these authors, should be accepted and incorporated in the already confused and contradictory literature of pectic materials.

NEW YORK AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK

LITERATURE CITED

1. CARRÉ, H. M., and HAYNES, D. The estimation of pectin as calcium pectate. Biochem. Jour. **16**: 60–69. 1922. Many modifications of this method have been proposed.
2. ELWELL, W. E., and DEHN, W. M. Pectic content of plant materials. Plant Physiol. **14**: 809–816. 1939.

NOTES

Annual Election.—The results of the seventeenth annual election of the American Society of Plant Physiologists have just been announced by the Secretary, Dr. W. E. LOOMIS, Iowa State College, Ames, Iowa. His report names Dr. FRANK P. CULLINAN, U. S. Horticultural Station, Beltsville, Maryland, as president for 1940–41; and Dr. BERNARD S. MEYER, Ohio State University, as vice-president.

Dr. JOHN W. SHIVE, who has just retired from the presidency, was elected to the Executive Committee for a term of three years, and Dr. H. R. KRAYBILL, Purdue University, was reelected to the editorial board for a three-year period.

These newly elected officers hold very responsible positions, which will present many opportunities for service. It is a pleasure to extend congratulations and to express the hope that the duties may not seem too arduous. The cooperation of the entire membership is assured.

New England Section.—The 1940 meeting of the New England Section of the American Society of Plant Physiologists was held at Dartmouth College, Hanover, New Hampshire, on May 10–11. The usefulness and value of the sectional meeting is indicated by the fact that 20 institutions were represented in the gathering, and more than 100 investigators were able to exchange ideas and discuss their mutual problems. The voluntary submission of papers brought out 28 contributions, which were presented in four sessions. Dr. CHARLES J. LYON of Dartmouth was in charge of the arrangements, and also performed the pleasant duty of introducing President JOHN W. SHIVE of the A.S.P.P., who brought greetings from the national organization. The evening program was distinguished by an illustrated lecture by JACK DURRANCE (Dartmouth, '39) on his famous trip through India and the climbing of "K-2" in the Himalayas.

Of special value in all such gatherings are the friendly and eager discussions that take place informally outside the regular sessions. It is common experience to gain more from these informal exchanges than from the formal programs.

The officers for the coming year were elected as follows. Chairman, Dr. THOMAS G. PHILLIPS, University of New Hampshire; vice chairman, Dr. DOROTHY DAY, Smith College; and secretary treasurer, Dr. LINUS H. JONES, Massachusetts State College.

Charles Frederick Hottes.—At the sixteenth annual meeting of the American Society of Plant Physiologists held at Columbus, Ohio, it was voted by the Society to dedicate one of the numbers of PLANT PHYSIOLOGY

during 1940 to Dr. CHARLES FREDERICK HOTTES, who celebrates the seventieth anniversary of his birth on July 8, 1940. The July number is therefore dedicated to him in honor of this occasion. Dr. HOTTES, son of FREDERICK and BARBARA (DATHAN) HOTTES, was born at Mascoutah, Illinois, a small town in St. Clair County. Following his education in the public schools, he entered the University of Illinois, from which institution he was graduated with a B.S. degree in 1894. He continued work in the graduate school at Illinois with the aid of a fellowship, and was awarded the M.S. degree in 1895. His excellent work led to his appointment to an assistantship in the Department of Botany of his alma mater, a position he held for three years. In August, 1895, he was married to Miss FLORA GUTH, also of Mascoutah. Their only child, a daughter, FLORA EMILY, lives at Kenosha, Wisconsin, where she is engaged in library work. During these early years, those who aspired to the doctorate in botany were accustomed to go to European universities. Dr. HOTTES chose to go to Bonn, where he received the M.A. and Ph.D. in 1901.

Returning to the University of Illinois, he was appointed to an instructorship for one year (1901–02) and became assistant professor in 1902, professor in 1913, consulting plant physiologist to the Department of Agronomy in 1923, and finally head of the Department of Botany in 1928, a position he held for 10 years, until his retirement. His connection with the University of Illinois as student and professor extended through a period of sightly less than a half century.

Dr. HOTTES is noted for his studies of cellular physiology and experimental cytology, and for his ingenuity in developing apparatus for the control of cellular environment, and for control of general environmental conditions. From his studies he has accumulated a large amount of valuable information, much of which unfortunately is still unpublished. Many monographs and books would be required to record the detailed results of his work; but he has refrained from publication, a serious loss to all of us who need to know what he knows about cellular responses.

One quality Dr. HOTTES possesses which makes him a rare teacher—his contagious enthusiasm for his subject. He held his classes spellbound by his masterly presentation of things not to be found in books. He could illustrate his lectures, and illuminate the discussions, with deep personal experiences and unique observations which gave distinction to his teaching.

Many students were attracted to the field of botany and to plant physiology because of his abiding enthusiasm for both plant physiology and students. He also gave unstintingly of time and energy to community interests and problems, and was always available to those who sought his services in a public enterprise.

He was a member of the American Society of Plant Physiologists very shortly after it was organized, and published a paper, *Studies in Experi-*

mental Cytology, in PLANT PHYSIOLOGY in 1929. At the Atlantic City meeting in 1932, he was honored by the Society as the eighth recipient of the CHARLES REID BARNES life membership award in recognition of his lifelong services to plant physiology, an honor richly merited.

The portraits reproduced in this number of PLANT PHYSIOLOGY were obtained through the kind cooperation of Professor JOHN T. BUCHHOLZ, Dr. HOTTES's successor as head of the Department of Botany at the University of Illinois. The portrait in plate IV is reproduced from a photograph of a painting which was presented to the University of Illinois by his former students at the time of his retirement. The original painting hangs in the reading room of the Natural History Building. This photograph scarcely does the painting justice. It represents the serious professor.

The photograph from which plate V was made dates from about 1935, and shows him as genial companion and friendly counsellor of students at the zenith of his career. It is a pleasure to be able to share these fine portraits with our readers.

On behalf of all members of the Society, and all of Dr. HOTTES's legion of friends, we extend to him and to his family most cordial greetings and good wishes on the happy occasion of his seventieth birthday anniversary, for long continued health and prosperity.

Rodney Howard True.—Again death has invaded the ranks of the life members of the American Society of Plant Physiologists. Everyone who knew him will feel keenly the loss of Dr. RODNEY HOWARD TRUE, who died at Philadelphia on April 8, 1940, in his 74th year. Dr. TRUE was an early member of the Society, and was the sixth recipient of the CHARLES REID BARNES award. This honor was bestowed upon him at the Cleveland meeting in 1930. A brief biography of TRUE was published in PLANT PHYSIOLOGY, volume 6, 197–198, 1931, following the award, and again in volume 12, 223, 1937, in honor of the seventieth anniversary of his birth, which occurred on October 14, 1936. A fine portrait of Dr. TRUE adorns the January, 1937, number of our journal. It is not necessary to add many details of his life at this time. He was deeply interested in the broader aspects of science and the history of science; and as long as he was physically able, he was active in the affairs of the American Association for the Advancement of Science. He is remembered especially for his work as chairman of the Committee of One Hundred.

The last public service which he rendered to the Society was in connection with the 15th award of the BARNES life membership. He was chairman of the award committee, and those who were present at the annual dinner at Richmond will recall the evident satisfaction with which he announced

that the life membership had been awarded to Dr. LUDWIG JOST of Heidelberg.

Dr. TRUE had suffered from heart ailments for a number of years, and his activities had been hampered by them; but he did not retire as emeritus professor of botany at the University of Pennsylvania until 1937, when he was in his 71st year. It was the increasing severity of the heart complications which finally caused his death. To his wife and other members of his immediate family we express our deep sympathy.

Annual Review of Biochemistry.—Volume IX of this valuable annual has been received, and again we commend it to our readers as a volume of extraordinary value. Some of the reviews cover the same regions of biochemistry as were covered in 1939, as *Biological Oxidations and Reductions*, and *Proteolytic Enzymes*. A number of them cover fields that were not reviewed last year. The new reviews, are, however, presented by different authors. Last year DIXON covered the field of oxidations and reductions; this year the advances are summarized by K. G. STERN. By changing authors, no one individual is overworked, and the interpretations have no tendency to become stereotyped.

The 1940 volume, 744 pages, contains 26 reviews, well distributed, and of about equal interest to plant and animal investigators. Of particular interest to plant physiologists are such reviews as *Plant Pigments*, by G. MACKINNEY; *Aspects of Inorganic Metabolism in Plants*, by C. B. LIPMAN; *Organic Acids of Plants*, by H. B. VICKERY and G. W. PUCHER; *Soil Microbiology*, by S. A. WAKSMAN; and *Biochemistry of the Lower Fungi*, by H. RAISTRICK. Most of the other reviews contain material of most general physiological applicability. They have been carefully prepared, and are worthy of careful reading by anyone who desires to keep up with the march of progress in biochemistry.

We recommend it most highly for public, institutional, and personal libraries. The bibliographies are valuable in themselves, and the work is a challenge to anybody's $5.00 bill. No greater value is to be obtained anywhere. It is a standard work, worthy of the support of all physiologists. Orders will be filled by Annual Reviews, Inc., Stanford University, California.

Sulphur Dioxide.—A comprehensive investigation of sulphur dioxide injury to vegetation has been in progress under the auspices of the National Research Council of Canada, for about a decade. The work was undertaken as a fact-finding study of injuries claimed to have been caused to vegetation in the area surrounding the great industrial plants of the Consolidated Mining and Smelting Co. at Trail, British Columbia.

The report of the investigations has been published with the title *Effect of Sulphur Dioxide on Vegetation.* The first half of the report concerns the field studies on the quantity of SO_2 found in the atmosphere near this industrial area; the symptoms of injury on forest and crop plants; the sulphur content of the vegetation in relation to the sulphur dioxide content of the air; hydrogen ion concentration, base exchange capacity, and sulphur content of the regional soils; hydrogen ion concentration and sulphate content of water supplies; and the effect of SO_2 on the diameter increment of conifers. This section of the work is profusely illustrated from photographs showing the characteristic injuries to leaves when the sulphur dioxide exceeds the tolerance of the vegetation. There are 24 excellent plates, two in colors which show the injuries found on needles of the yellow pine and Douglas fir. These conifers are among the more sensitive species.

The second half of the report deals with fumigation experiments. The plots and apparatus used in the experimental investigations are described, and the succeeding chapters discuss fumigation experiments on conifers in their natural habitat; on transplanted conifers; the effects of environmental factors on the susceptibility of barley and alfalfa to sulphur dioxide; stomatal behavior of fumigated alfalfa; experiments on the yield of barley and alfalfa; the effect of fumigation on some chemical constituents of barley, wheat, and alfalfa; and CO_2 assimilation and respiration of alfalfa under the influence of SO_2. The final chapter is a summary, chapter by chapter, of the entire report.

This monograph is a valuable contribution to the subject of SO_2 injuries to vegetation. It contains scientific and practical information that will be extremely useful to investigators and to industrial management. It is bound in handsome blue cloth, and because of the large number of text figures, plates, and the colored plates, it is an expensive publication. Nevertheless, it deserves a wide distribution among plant physiologists, and among our technical libraries. The price quoted is $15.00 per copy; it is obtainable through the National Research Council of Canada at Ottawa.

Methods of Enzyme Research.—The first section of an important compilation of research methods applicable to enzyme chemistry has been received from the press of Georg Thieme, Leipzig. It is appearing under the title *Die Methoden der Fermentforschung.* This first "Lieferung" occupies 172 pages, 8×11. The general introduction, 8 pages, defines terms and presents a short historical survey of the field.

The first "Hauptteil" is a general section, which begins with a consideration of the substrates; and first of all, with the preparation, characteristics, and the investigation of important substrates, intermediates, and end products. The substrates of the ester-splitting enzymes are considered at

the outset, including such as the substrates of lipases; naturally occurring glycerides; acetylcholine; gallotannin and other substrates of tannase; chlorophyll; simple esters of phosphoric acid; inosite phosphoric acid; phosphatides and their hydrolytic products; and simple esters of sulphuric acid. Each of these topics is presented by a collaborating author. The section on chlorophyll is by HANS FISCHER, Munich; and the one on glycerides by KARL HUGO BAUER, Leipzig, etc. The main editors of this elaborate work are Dr. EUGEN BAMANN, Tübingen, and Dr. KARL MYRBÄK, Stockholm.

The last 57 pages of this "Lieferung" are occupied by four papers on biologically important carbohydrates and glycosides. The sugars are discussed by E. L. HIRST and S. PEAT of England; preparation of natural heterosides, by J. RABATÉ, Paris; synthetic glycosides, by HORST ELSNER, Berlin; and thioglycosides, by FRITZ WREDE, Berlin.

It is not known at the present time just what the cost of this valuable work may be, or how rapidly succeeding parts may become available. This first section, 172 pages, is listed at RM 18, and a general price of RM 1.6 for each 16-page folder has been set. There will be about 220 of these, which indicates well over 3000 pages in the completed work. About 6000 references in the text. Purchasers are reminded that those who order the first section are considered to be obligated to the publisher to accept and pay for the entire series of "Lieferungen." Separate sections are not purchasable.

Attention is also called to the price reduction of 25 per cent. for foreign orders, when the payments are made in accordance with stipulated conditions (Devisen oder Freimarke).

The work is most highly recommended to all investigators and to all establishments concerned in enzyme research of any kind. Every institutional library which caters to investigational needs should subscribe for it as an indispensable item. Orders should be placed with the publisher, Georg Thieme, Publisher, Leipzig.

Annual Review of Physiology.—The second volume of the *Annual Review of Physiology* contains twenty reviews, each one dealing with the recent progress of research in some special phase of animal or human physiology. A few representative titles are as follows: Developmental physiology; respiration; the digestive system; the lymphatic system; energy metabolism; bioelectric potentials; endocrine glands; mammalian reproductive organs; exercise; and physiological psychology.

These reviews have been carefully prepared, and give an adequate account of the recent additions to our knowledge in the fields presented. They are valuable because of the time saved for investigators, teachers, and students who must obtain in a short time a clear picture of the front-line situa-

tions. This annual has taken its place along with the *Annual Review of Biochemistry* as an invaluable aid in the progress of physiology. The editors and authors deserve much credit for this excellent contribution to science. The volume contains 501 pages, and is sold at $5.00 per copy by Annual Reviews, Inc., Stanford University, California.

Trees and Shrubs.—Horticulturists and plant physiologists must often use cultivated varieties of trees and shrubs in their investigations. Taxonomic problems frequently confront the worker who desires to state definitely what variety he used for his studies. The second edition of Dr. ALFRED REHDER's *Manual of Cultivated Trees and Shrubs*, which was released on June 4, 1940, by the Macmillan Co., will be a welcome aid in solving these taxonomic difficulties. It offers a synopsis of the orders and families covered, and an analytical key to the families and aberrant genera. The main body of the manual, of course, provides the names and descriptive characterizations of a very large number of cultivated woody plants. This edition includes many new introductions and hybrids, and also incorporates changes made desirable by changes in the rules of nomenclature adopted by the Botanical Congresses at Cambridge and Amsterdam.

The revision of such a work requires a vast amount of labor, and Dr. REHDER has rendered a great service to his fellow botanists who owe him a debt of gratitude for this excellent source of information. The price quoted for the new edition is $10.50 per copy. Orders may be sent to the Macmillan Co., New York.

Vol. 15 No. 4

PLANT PHYSIOLOGY

OCTOBER, 1940

A STUDY OF THE PIGMENTS PRODUCED IN DARKNESS BY CERTAIN GREEN ALGAE

JACK MYERS

(WITH FIVE FIGURES)

An observation which has become well established is that certain green plants (*e.g.*, some of the green algae) are able to produce their chlorophyll in the dark. The experimental data characterizing the pigments so produced, however, are indeed meager. It therefore has seemed advisable to reinvestigate the pigments formed by such plants in the dark with the improved spectroscopic methods now available. In the first place there is no conclusive evidence that tells whether or not chlorophyll *b* is formed in darkness. Secondly, it is to be hoped that a study of these rather exceptional cases of chlorophyll formation in the dark may well be a foothold in the study of the general process of pigment development. And thirdly, it is conceivable that spectroscopic differences between "light" and "dark" pigments might be found which could later be correlated with the beginnings of photosynthesis.

This paper is therefore a report of the data so far obtained in a spectroscopical and physiological study of the pigments formed by green algae in the dark.

Although it had previously been noted (*e.g.*, HEINRICHER (7), SCHIMPER (15), and KLEBS (8)), the first unqualified demonstration of the greening by algae in the dark seems to have been made by BEIJERINCK (3) in 1890 on a pure culture of *Chlorosphaera limicola*.[1] A later paper by the same worker reported (4) that various pure cultures of green algae (including *Chlorella vulgaris, Stichococcus bacillaris*, and *Scenedesmus acutus*) had been grown for several years in absolute darkness. The cultures developed quantities of green cells which again became autotrophic when brought into light. The work of ARTARI (1) also demonstrated the greening of algae and lichen gonidia in darkness. ÉTARD and BOUILHAC (6) compared spectroscopically

[1] A colored plate shows green cells that had been grown in the dark on a sucrose-peptone gelatin.

the alcoholic extracts of the blue-green alga, Nostoc, grown on sugar solution in darkness with extracts of green leaves. Their methods gave no significant differences between the two. RADAIS (14) made a somewhat more detailed spectroscopic study of the alcohol and carbon disulphide extracts of parallel light and dark cultures of *Chlorella vulgaris.* He obtained identical absorption spectra for the extracts of the two cultures. In addition to the "end absorption" below 511 mμ he observed three absorption bands with mean axes at 667, 618, and 577 mμ. His carbon disulphide extract gave bands with mean axes at 679, 659, 625, and 583 mμ. The occurrence of a distinct additional absorption band in the red at 659 mμ for the carbon disulphide extract suggests the presence of the *b* component. It is rather difficult, however, to see why the alcoholic extracts failed to show a similar band. And by analogy to the absorption curves in other solvents (*e.g.*, ZSCHEILE (25)) it seems rather surprising that a chlorophyll *b* peak would show up so clearly in this region unless the *b* component were present in an unusually high proportion.

DANGEARD (5) cultivated *Scenedesmus acutus* for eight years in darkness and found that it maintained its green color. The algae brought into light at the end of that time showed within five hours a photosynthetic activity (liberation of bubbles of oxygen). Unfortunately DANGEARD's technique of observing photosynthesis was neither rapid enough nor sufficiently quantitative. Considerable pigment changes might have taken place in less than five hours or might still have been going on at the end of that time (*cf.* SEYBOLD, 16).

While the available data are consistent with the view that the pigments formed in darkness by certain green algae are photosynthetically effective, in the light of more recent knowledge it has seemed advantageous to reinvestigate this problem with the improved methods of pigment study and photosynthesis measurement now available.

Since the completion of the experimental work of this paper, there has come to the author's attention the recent publication of VAN HILLE (22). Incidental to his study of the relation between rate of photosynthesis and chlorophyll content this worker studied *Chlorella pyrenoidosa* grown in darkness. He states that methanol extracts of cells grown in light and in darkness showed the same absorption spectra, although he includes no data and apparently worked only at wavelengths greater than those absorbed by the carotenoids. VAN HILLE also found for cells grown in darkness (p. 748) that "The readings per half-hour of photosynthetic measuring are constant from the beginning and show the normal time of induction (SMITH, 1937)." Again no data are included. Although VAN HILLE's findings have been duplicated, the present paper is considered justified by the more detailed and comprehensive data obtained.

Spectroscopy of the pigments

Three species of green algae were used in this study: *Chlorella vulgaris*, *Protococcus* sp., and *Chlorococcum* sp.[2] These were grown in pure culture on the agarized Detmer's solution diluted to ⅓ as recommended by MEIER (11) in flat one-liter medicine bottles with loose cotton plugs. Bacteriological technique was observed. Three parallel series were used: (1) in darkness with added organic nutrient; (2) in light with added organic nutrient; (3) in light without added organic nutrient. Added organic nutrient consisted of 0.5 per cent. dextrose and 0.2 per cent. peptone. Cultures in light were continuously illuminated by tungsten filament bulbs with Corning Aklo filters to reduce the infra-red. Intensity at the level of the cultures was 65 foot candles as recorded by the Weston photronic cell.

Common methods of extraction employing cold solvents proved ineffective. The use of hot solvents was considered dangerous in view of the labile nature of the pigments. A method has been devised which is a modification of the method used by MUDD and co-workers (13) for the extraction of labile bacterial antigens. A suspension of algal cells, washed off the agar surface, is placed in a metal ball-mill[3] and rapidly frozen around the inside rim by rotating the mill in a $-80°$ C. bath (dry ice in alcohol). Freezing is completed in less than one minute. The ball-mill is then quickly attached to a high vacuum line in series with a low temperature condenser held at $-80°$ C. The ice is thus rapidly sublimed off and drying is complete in 12 to 16 hours. The mill is then returned to the low temperature bath and two steel balls inserted. After an hour and a half grinding the bath is removed, 90 per cent. acetone introduced into the ball-mill, and grinding continued for another hour. The acetone extract is removed and the pigments transferred to ethyl ether. (Anesthesia ether, Malinckrodt, containing 2.5 per cent. alcohol was used throughout because of its very low peroxide content.)

Unfortunately the above procedure is not quantitative in that not all of the algal cells are crushed and extracted. Conditions are nearly optimal, however, for the preservation of labile and easily oxidizable cellular materials such as the chlorophylls and carotenoids.

Spectral absorption curves on the ether solutions have been determined, using the photoelectric spectrophotometer previously described by MILLER (12) and observing the precautions which he suggested. At some time during each run the calibration curve for prism setting was checked ($\pm$ 2 Å)

[2] Identified by Professor FELIX MAINX of the German University at Prague. Obtained through the courtesy of Dr. C. E. SKINNER by whom they had been isolated from soil.

[3] Kindly provided by the Department of Veterinary Science, the University of Minnesota.

against the 6402 Å neon line. The slit-widths used varied from 0.04 mm. at 4000 Å and 0.02 mm. at 4200 Å to about 0.002 mm. at 6000–6800 Å. In the absorption curves to be presented intensity of absorption is designated merely as $\log \frac{I_0}{I_x}$ according to Beer's Law, since concentrations are not known. The wavelength axes are in Ångström units.

A method previously used by STRAIN (20) has been followed in order to separate into two groups the chlorophyll and carotenoid pigments. This is demonstrated by the curves of figure 1 for the extracted pigments from

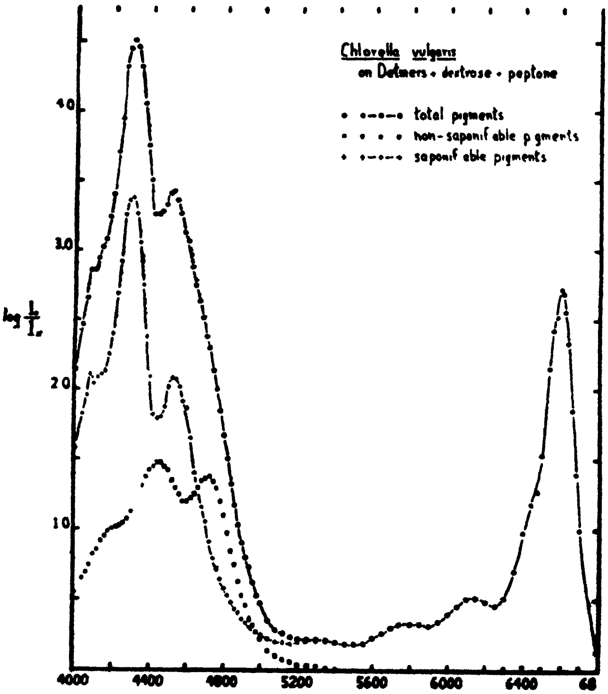


FIG. 1. Absorption spectra of pigments in ethyl ether + 2.5 per cent. alcohol extracted from *Chlorella vulgaris* grown in darkness. Curves refer to equal aliquots of the original extract. For further explanation see text.

Chlorella vulgaris grown in darkness. The upper curve describes the light absorption of the total pigments. If an equal or known aliquot of this ether solution is saponified and the non-saponifiable pigments taken up in ether, the lower curve is obtained. This may be considered the absorption curve for the carotenoid pigments. If the lower curve is now subtracted from the upper curve, point-by-point in the original data, the intermediate curve is obtained. This may be considered the absorption curve of the saponifiable pigments, *i.e.*, of the chlorophylls.

The method of course depends upon the assumption that the saponification removes all the chlorophylls but destroys none of the carotenoids. In my hands the common cold saponification has failed to remove all the chlorophylls, as shown by residual light absorption at 6400–6600 Å. A hot saponification has been required.[4] Comparison of hot and cold saponification on equal aliquots of the pigments of *Chlorococcum* sp. grown in the dark is given in figure 2. Intensity of absorption is significantly higher after the

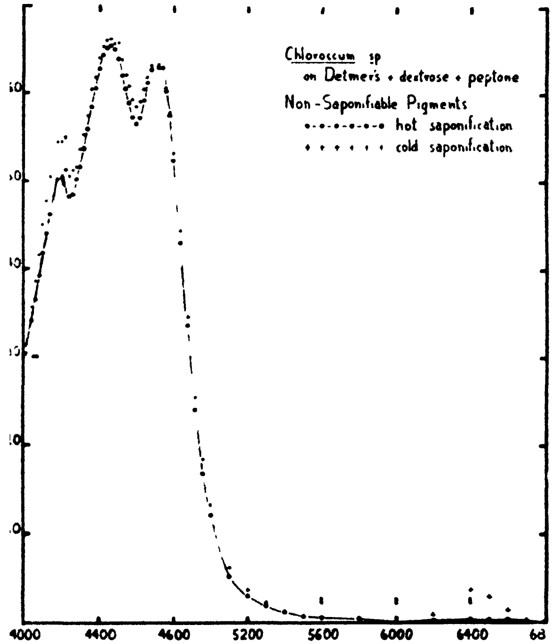


FIG. 2. Absorption spectra of the pigments remaining after hot and after cold saponifications. Pigments originally extracted from *Chlorococcum* sp. grown in darkness.

cold saponification only in those spectral regions where the chlorophylls show high absorption.

Figure 3 shows the absorption curves for the total pigments extracted from *Protococcus* sp. grown under three different conditions. For two of these curves all values of $\log \frac{I_0}{I_x}$ have been multiplied by a factor so chosen

[4] To 4 ml. of 30 per cent. KOH in methanol refluxing over a water bath, 5–10 ml. of the ether solution are added and refluxing continued for 3 minutes. The total solution is then poured into separatory funnel containing ice water and about 10 ml. of ether. The resulting ether solution of carotenoids is washed 5 times with water, separated, and made up to the desired volume. For cold saponification the same time, quantities, and reagents were used and the entire procedure carried out in a separatory funnel.

as to give identical values for the intensity of absorption at 6600 Å on all curves. This merely brings the curves together for ease of comparison. The corresponding absorption curves for the chlorophyll pigments, obtained as described above, are presented in figure 4. Agreement is rather striking, especially through the longer wavelengths of the spectrum. Noticeable variations occur in the height of the bands in the blue: the 4520 Å peak of chlorophyll *b* and the 4100 Å and 4300 Å bands principally due to chlorophyll *a*. These variations may be accounted for in part by variations in the ratio of *a* to *b*.

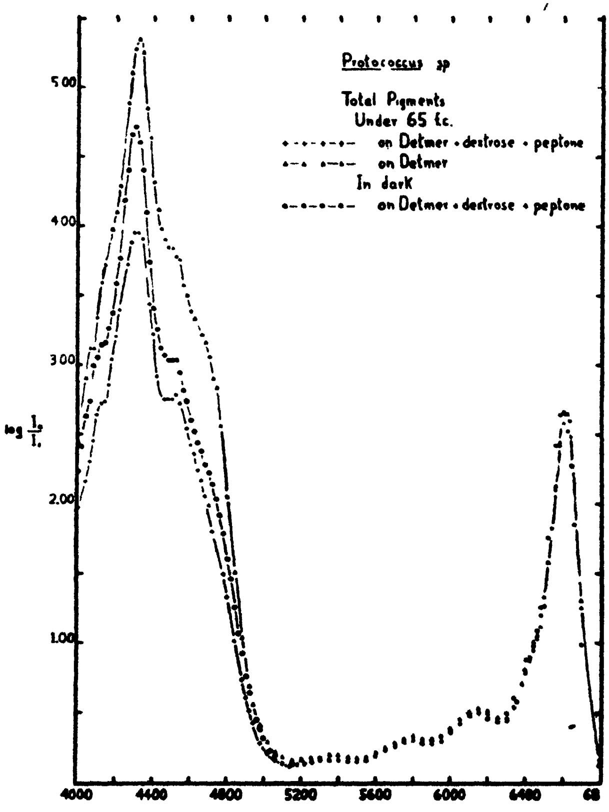


FIG. 3. Absorption spectra of the total pigments extracted from *Protococcus* sp. grown under three different conditions.

Comparison of the chlorophyll curves has been made with the absorption curves of the isolated components obtained by ZSCHEILE (25) on the same type of instrument. In general the curves of figure 4 (for ether + 2.5 per cent. alcohol) show a displacement of the peaks toward the red of about 10 to 25 Å. Because of this shift a quantitative analysis of the curves for percentages of the components *a* and *b* by the method of ZSCHEILE (25), and

based on his curves for the chlorophylls in ether solution, is impossible. Only anomalous results can be obtained.

The presence of the well-defined 4520 Å band in all the curves of figure 4 as well as in the intermediate curve of figure 1 makes it clear that chlorophyll *b* (or something very similar) is formed in the dark by these cultures of Protococcus and *Chlorella vulgaris*. And inspection of the curves of figure 4 shows that in Protococcus the chlorophyll *a*: *b* ratio is at least of the same

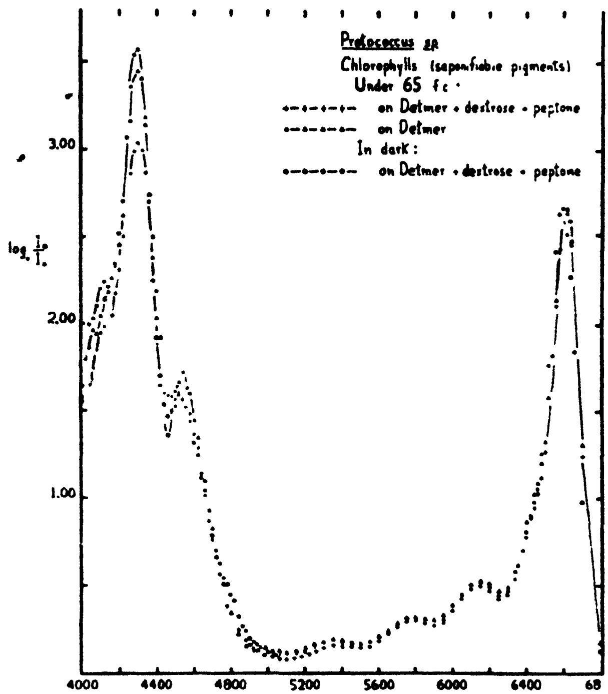


FIG. 4. Absorption spectra of the chlorophylls produced by *Protococcus* sp. grown under three different conditions.

order of magnitude for cells grown in light or in darkness. The formation of chlorophyll *b* in the dark has been confirmed by the appearance of distinct and characteristic chlorophyll *b* zones on sucrose chromatograms prepared according to the method of SEYBOLD and EGLE (17). Comparison of the curves of figures 3 and 4 indicates that the carotenoid : chlorophyll ratio varies considerably under the different conditions of growth. More detailed examination of the carotenoids should be made to determine whether these variations are qualitative or merely quantitative in nature. Further chromatographic work planned for the study of the yellow pigments has not as yet been carried out.

Development of photosynthetic activity

It was advisable at this point to consider the development of photosynthetic activity in algal cells which had previously grown only in darkness. Essentially this involved a comparison of the induction periods of cells grown in darkness and in light. Measurements were made by the familiar Warburg technique, using one illuminated flat-bottom vessel and a non-illuminated thermobarometric control. The volume of the experimental flask was 14.22 ml. to the level of Brodie fluid in the manometer, as calibrated with mercury. When used as described below 1.0 mm. increase in pressure corresponds to an oxygen evolution of 0.56 cu. mm.

The constant-temperature bath was held at 26° C. ± 0.01° as checked by a Beckmann thermometer. Light was provided by an optical system mounted horizontally beneath the bath. The light from a projection bulb, suitably condensed by two lenses, was reflected vertically up through the glass bottom of the bath by a concave mirror. The area of the light beam in cross-section at the level of the experimental flask was large enough so that the flask was always illuminated during the three-cm. amplitude of its shaking cycle. This arrangement was designed to give very high light intensities, since previous work [WARBURG (**24**), SMITH (**19**), MCALISTER (**9**)] indicates that the induction period is longer, and therefore more easily measurable, under higher intensities. At very high light intensities, however, (about 5,000–20,000 f.c.) an inhibition of photosynthesis was observed. A study of this inhibition is to be reported elsewhere. For measurement of the induction period an intensity of 2,200 f.c. was used.

Preliminary results indicated that more consistent results could be obtained for cells grown in liquid culture than for those grown on solid media as in the pigment study. It was also found that *Chlorella vulgaris* was much better adapted for this part of the study than the other algae available. *Protococcus* sp. could not be centrifuged out cleanly and showed such low rates of photosynthesis that it was more difficult to work with. Cultures were grown in 500-ml. Erlenmeyers with a current of air bubbled through. To obtain darkness, flasks were wrapped in photographic light-proof paper and kept in covered iron pails. Cultures grown in light were placed uniformly around a 5.5-cm. water jacket surrounding a 300-watt bulb. The intensity at the illuminated side of the flasks was about 450 f.c. Sterile precautions were observed only when glucose was added to the nutrient solution, although microscopic tests for purity were made in all cases.

Measurements were made with the cells suspended in the potassium carbonate-bicarbonate buffer corresponding to the sodium carbonate-bicarbonate buffer no. 9 of WARBURG (**23**) (0.015 M K_2CO_3, 0.085 M $KHCO_3$). Immediately before an experimental run the cells were centrifuged out of the nutrient solution, taken up in the buffer, and centrifuged out again in

a graduated tube. To the packed cells fresh buffer was added to give a suspension in which 1.0 ml. contained 0.05 ml. of cells. The suspension was then kept in the dark and aliquot portions withdrawn in the dark by an automatic pipette. When cells were grown in darkness, all further operations except the brief reading of cell volume were performed in the dark.

One ml. of cell suspension was added to 7.2 ml. of buffer in the experimental flask. The flask and manometer were placed in position without illumination and about five minutes allowed for adjustment to equilibrium. Four or five consecutive five-minute readings were taken to establish the course of respiration. Light was then turned on for ½ minute. Respiration in the succeeding dark period was again established by at least four readings. This procedure was repeated with light exposures of 1, 1.5, 2, 3, and 4 minutes, using a fresh batch of cells for each exposure. The displacement between the respiration curves before and after the light exposure is a measure of the amount of photosynthesis which took place. This graphical method is illustrated by the upper curves of figure 5. It is an "integrational" method necessitated by the lag in the Warburg instrument.[5]

The method is of course limited to the accuracy of extrapolation of the respiration curves. Occasionally a deviation in the rates of respiration before and after illumination was observed, but never of more than about 5 per cent. It is recognized that such a variation introduces a possible source of error in the extrapolation. Several preliminary experiments seemed to indicate that more consistent results were obtained when a fresh aliquot of cells was used for each time interval and when 0.5 per cent. dextrose was added to the stock suspension kept in the dark. These conditions were therefore observed throughout.

The induction curves obtained for *Chlorella vulgaris* as described above are shown in figure 5. The close coincidence of the curves for cells grown with 0.5 per cent. dextrose, with and without light, clearly answers the problem of this investigation. *The pigments produced by this green alga in darkness are adequate for the process of photosynthesis.* Cells grown in the light without dextrose (upper curve) attain a somewhat higher photosynthetic rate and seem to have a somewhat longer induction period than cells grown with dextrose. The *induction loss*, however [extrapolated intercept on the photosynthesis axis, *cf.* McALISTER (**9, 10**)], is practically identical for all three lots of cells.

In considering the above data as a further description of the induction phenomenon, a possible limitation of the integrational method must be taken into account. McALISTER (**10**) has demonstrated that after high rates of photosynthesis there is an appreciable "pick-up" of CO_2 after the plant is

[5] This is essentially the same method previously used by WARBURG (**24**), VAN DER PAAUW (**21**), and SMITH (**19**).

darkened. Making the reasonable assumption that there is also a continued evolution of O_2 by a plant on darkening, comparable to the "pick-up" of CO_2, he has suggested that there may be an inherent error in the integrational method used here. What is probably measured for each period of illumination is the actual amount of photosynthesis during illumination plus the "pick-up" (in this case the extra evolution of oxygen) on darkening. Each point on the induction curve is therefore too high by an amount equal to the "dark pick-up" involved in its determination. So an induction curve measured by the integrational method is probably somewhat in error in regard to its shape and *induction loss.* From the characteristics of the "dark pick-up," however, it seems unlikely that the indicated length of the induction period will be appreciably in error.

The papers of McAlister (**10**) and Aufdemgarten (**2**) have shown that the induction period is a function of the preceding dark rest when photosynthesis is measured by the rate of CO_2 uptake. Aufdemgarten (**2**) states that the induction period in Stichococcus is lengthened by increasing dark rests of up to fifteen minutes, beyond which no further effect was noted. McAlister's (**10**) more comprehensive data for wheat show a rapid increase in *induction loss* with dark rests increasing up to about one minute, followed by a more slowly progressing effect up to about eight hours. These papers had not yet appeared when the experimental work was done. No attempt was made to control the preceding dark rest, which varied from about two to eight hours during each run for cells grown in light. The experimental points, however, were not obtained in any definite order and any appreciable change in the true induction period during this time would hardly have permitted the points for the two, three, and four minutes illumination to fall on a straight line. Of course, the final straight line may not be truly constant in slope. Indeed, in continuous light all three batches of cells showed photosynthetic rates increasing slowly with time and always slightly higher than the final rates indicated by the curves in figure 5. But following the usual interpretation, the induction period is clearly over somewhere between one and two minutes of illumination. Certainly dark rests of more than two hours could have had little if any additional effect on the characteristics of the induction period in *Chlorella vulgaris.*

It is also of interest to compare the induction curves (fig. 5) with those obtained by other workers using the same method, which is based on the rate of evolution of oxygen. Smith (**19**), using a preceding 30-minute dark rest, found for Cabomba induction periods of about two to five minutes depending on the light intensity and CO_2 concentration. Van der Paauw's (**21**) curves for Hormidium show an induction of 1½ minutes at 26° C. (length of dark rest not stated). Warburg (**24**) observed for *Chlorella vulgaris* an induction period of 1½ minutes after a five-minute dark rest. His conditions

of light intensity, CO_2 concentration, and temperature were almost identical with those maintained in the present study. The induction period obtained by WARBURG after a five-minute dark rest is identical with that shown in figure 5 for the same organism after an infinitely long dark rest. This is also in line with WARBURG's data for one- to five-minute periods of intermittent light.

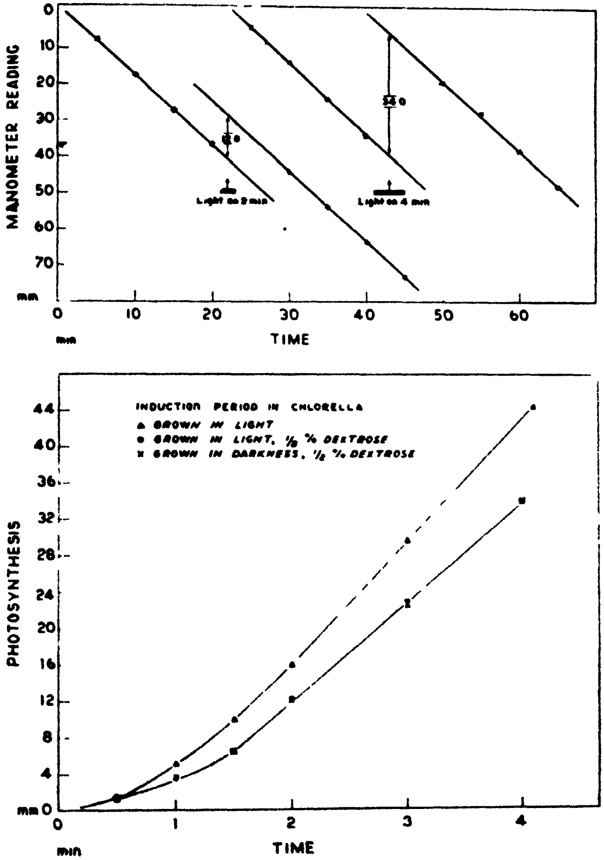


FIG. 5. The induction period in *Chlorella vulgaris*. Ordinates are readings from the Warburg manometers in mm.; multiply by 0.56 to obtain cu. mm. of O_2. The upper set of curves describes the method by which each point in the lower set of induction curves was obtained. For further explanation see text.

Any detailed study of the effect of the preceding dark rest on the induction period was not a planned part of this investigation, and the results are certainly not critical in this respect. However, after comparison of the data of WARBURG and of this paper on one hand with those of McALISTER or of AUFDEMGARTEN on the other, it seems that the induction in O_2 production

and the induction in CO_2 uptake are not equally dependent upon the preceding dark rest, and are therefore probably dissimilar in other respects.

Summary

1. The pigments produced by *Protococcus* sp. and *Chlorella vulgaris* in darkness have been studied with a photoelectric spectrophotometer and compared with the pigments produced in light. For this purpose a method of extraction not previously used in pigment work has been described.

2. No significant qualitative differences have been found between the chlorophyll pigments produced in light and in darkness.

3. The induction periods for cells grown in light and in darkness have been studied by the WARBURG technique for a strain of *Chlorella vulgaris*. Only slight differences have been found between the induction curves of cells grown in darkness and in light.

4. The pigments produced by this strain of *Chlorella vulgaris* in darkness are adequate for the process of photosynthesis.

5. From a comparison of the data obtained with other data in the literature on the induction period it has been suggested that there is a dissimilarity between the induction in O_2 evolution and the induction in CO_2 uptake.

The author gratefully acknowledges the helpful advice and criticism of Dr. GEORGE O. BURR, Dr. E. S. MILLER, and Dr. C. E. SKINNER.

THE DEPARTMENT OF BOTANY
UNIVERSITY OF MINNESOTA

LITERATURE CITED

1. ARTARI, A. Über die Bildung des Chlorophylls durch grüne Algen. Ber. d. bot. Ges. **20**: 201–207. 1902.
2. AUFDEMGARTEN, H. Zur Kenntnis der sogenannten Induktionsvorgänge bei der Kohlensäureassimilation. Planta **29**: 643–678. 1939.
3. BEIJERINCK, M. W. Culturversuche mit Zoochlorellen, Lichengonidien und anderen niederen Algen. Bot. Zeit. **48**: 724–771; 781–785. 1890.
4. ————. Notiz über *Pleurococcus vulgaris*. Cent. f. Bakt. II **4**: 785–787. 1898.
5. DANGEARD, A. P. Observations sur une algue cultiveé à l'obscurité depuis huit ans. Compt. Rend. Acad. Sci. (Paris) **172**: 254–260. 1921.
6. ÉTARD, A. and BOUILHAC, M. Presence des chlorophylles dans un Nostoc cultivé à l'abri de la lumiere. Compt. Rend. Acad. Sci. (Paris) **127**: 119–121. 1898.
7. HEINRICHER, E. Zur Kenntnis der Algengattung Sphaeroplea. Ber. d. bot. Ges. **1**: 433–450. 1883.

8. KLEBS, G. Beiträge zur Physiologie der Pflanzenzelle. Ber. d. bot. Ges. **5**: 181–188. 1887.
9. MCALISTER, E. D. Time course of photosynthesis for a higher plant. Smithsonian Misc. Coll. **95**: no. 24. 1937.
10. ———. The chlorophyll-carbon dioxide ratio during photosynthesis. Jour. Gen. Physiol. **22**: 613–636. 1939.
11. MEIER, F. Effects of intensities and wavelengths of light on unicellular green algae. Smithsonian Misc. Coll. **92**: no. 6. 1934.
12. MILLER, E. S. A precise method, with detailed calibration, for the determination of absorption coefficients; the quantitative measurement of the visible and ultra-violet absorption spectra of alpha carotene, beta carotene, and lycopene. Plant Physiol. **12**: 667–684. 1937.
13. MUDD, S., SHAW, C. H., CZARNETZKY, E. J., and FLOSDORF, E. W. A low-temperature ball-mill for the liberation of labile cellular products. Jour. Immunol. **32**: 483–489. 1937.
14. RADAIS, M. Sur la culture pure d'une algue verte; formation de chlorophyll à l'obscurité. Compt. Rend. Acad. Sci. (Paris) **130**: 793–796. 1900.
15. SCHIMPER, A. F. W. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde. Jahrb. wiss. Bot. **16**: 1–247. 1884.
16. SEYBOLD, A., and EGLE, K. Lichtfeld und Blattfarbstoffe. II. Planta **28**: 87. 1938.
17. ———, and ———. Zur chromatographischen Methode der Blattpigmente. Planta **29**: 114–119. 1938.
18. SMITH, E. L. The influence of light and carbon dioxide on photosynthesis. Jour. Gen. Physiol. **20**: 807–830. 1937.
19. ———. The induction period in photosynthesis. Jour. Gen. Physiol. **21**: 151–163. 1937.
20. STRAIN, H. H. Leaf xanthophylls. Carnegie Inst. Washington. Pub. no. 490. 1938.
21. VAN DER PAAUW, F. The indirect action of external factors on photosynthesis. Pp. 595–598. Amsterdam, 1932.
22. VAN HILLE, J. C. The quantitative relation between the rate of photosynthesis and chlorophyll content in *Chlorella pyrenoidosa*. Rec. trav. bot. néerl. **35**: 682–757. 1938.
23. WARBURG, O. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. Biochem. Zeitschr. **100**: 230–270. 1919.
24. ———. Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. II. Biochem. Zeitschr. **103**: 188–217. 1920.

25. ZSCHEILE, F. P., Jr. An improved method for the purification of the chlorophylls A and B; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. Bot. Gaz. **95**: 529–562. 1934.

USE OF TENSIOMETERS IN REGULATING WATERING IN FOREST NURSERIES[1]

J. H. Stoeckeler and Einar Aamodt

(WITH SIX FIGURES)

Introduction

Soil moisture is one of the most important factors influencing physiological processes, such as growth of plants, their development as regards root and top, effect on drought resistance, frost hardiness, and dormancy. These physiological effects have a special importance in large-scale forest nursery production where the maintenance of a proper water supply in the soil is one of the controlling factors in the production of high-quality planting material. Methods of determining soil moisture are still susceptible of improvement and any advances made in this field are of practical significance.

The regulation of watering in forest nurseries has always been a problem, even in those nurseries equipped with overhead irrigation systems. Some of the difficulty has come about because of differences in soil, species, topography, windbreak protection, shade, age class, and relative density at which the stock is grown. It has been observed that first-year conifer seedlings need light but frequent watering, whereas older stock needs fewer but heavier irrigations. The smaller seedlings require more frequent watering because of their limited depth of rooting, and because their thin cortex is not as good an insulator against sun injury as is the thicker bark of seedlings two or more years old. The sandier and the more exposed parts of the nursery need more frequent irrigation than the rest of the nursery. Seedlings under half shade require less than those without shade.

Although irrigation will be based largely upon the experience of the nurseryman, his judgment will be aided materially by some instrumental method of measuring the moisture content of the soil.

The chief disadvantages of many of the available instruments have been that they are difficult to construct and operate or too expensive to purchase in sufficient numbers to get adequate coverage in the nursery. Oven-drying of soil samples is considered impracticable owing to the time involved in taking samples and also to the fact that results cannot be obtained until about 24 hours after sampling. There are, of course, several other methods, including the alcohol-burning method described by Bouyoucos (1) and auto-irrigators or soil points (6), which provide a means of getting results in an

[1] Contribution from Lake States Forest Experiment Station, University Farm, St. Paul, Minnesota. Maintained by the U. S. Department of Agriculture, Forest Service, in cooperation with Division of Forestry, University of Minnesota.

hour or less. These have been proved to be of practical value in forest nurseries, but are relatively time consuming.

One of the instruments developed in recent years which shows considerable promise in gauging moisture conditions in soils is the "tensiometer."[2] This device measures capillary pull of soils at various moisture contents. The so-called capillary pull, in simple terminology, represents the amount of force with which the wedges or lenses of capillary water between soil particles are held against extraction. In the usual type of tensiometer this force is expressed in centimeters or millimeters of mercury and is determined by attaching to a mercury manometer a fine-textured porous clay pot filled with boiled distilled water. This pot is buried in the soil at the desired depth and the rise in the mercury column is observed. The drier the soil, the higher will be the mercury lift observed on the manometer. If a series of readings is taken over a range of moisture conditions, it is possible to obtain a calibration curve for any specific soil at a given degree of compaction that shows the equivalent of a certain moisture content expressed in centimeters of mercury. Where a more rugged and durable type of instrument is needed, an industrial vacuum gauge may be substituted for the more fragile mercury manometer.

The one serious objection to tensiometers is that they will record a maximum pull of about 55 to 65 centimeters of mercury, and it has been shown for some of the fine-textured soils, such as clays, or clay loams, that this lift represents only about 70 to 85 per cent. of the moisture range in which plants will survive. Such heavy soils, however, are not ordinarily used for conifer nurseries, and lifts of 0 to 50 cm. of mercury represent practically the entire range of moisture conditions in which small conifers can survive and develop properly in sandy soils.

Tensiometers have been used on an experimental basis by a number of investigators. KORNEV (5) in 1921 devised one of the first tensiometers by making use of the method already described, although LIVINGSTON (6) mentions that similar experiments had been conducted as early as 1908 at the Desert Laboratory near Tuscon, Arizona.

LYNDE and DUPRÉ (7) devised apparatus in 1913 to study the capillary lift of soils at pressures ranging from 1.37 to 0.74 atmospheres, and concluded that capillary lift was limited by pressure of the atmosphere and that maximum lifts under one atmosphere of pressure could not exceed 10 meters, or 34 feet.

HEATH (2), in a rather intensive test, made up 30 of these instruments,

[2] In this paper the terms "tensiometer," "tonometer," and "moisture meter" are used synonymously. The term "tensiometer," recommended by L. A. RICHARDS, is undoubtedly the most descriptive and accurate in a scientific sense, but "moisture meter" is probably the better term for the layman.

varied the moisture content of the soil, and reported a good correlation of the manometer readings with the amount of water added.

HECK (3), with a modification of the tensiometer built by HEATH, successfully used such instruments as a guide in determining when it was necessary to irrigate sugar cane in Hawaii He reports that when the soil became dry enough to lift the mercury column 25 or 30 centimeters, active growth practically ceased and it was necessary to apply irrigation water to bring the soil moisture back to an optimum for continued rapid plant growth.

In a technical discussion of the relation of physical properties of soil to capillary behavior, HAINES (4) shows that a marked increase in pressure deficiency occurs when the soil is at 80 per cent. or less of saturation, and climbs sharply, to a point at 20 per cent. of saturation, from whence the curves flatten off. His work has a bearing on the use and calibration of tonometers, for he shows that the pressure-deficiency curve for rising moisture does not coincide entirely with that for falling moisture. This phenomenon is referred to as "hysteresis" and is further discussed in detail by SMITH (13).

This discovery has a practical bearing on the use of tensiometers in field setups because moisture content in the field, especially in the surface layers of soil, is generally slowly dropping, except during periods of replenishment by rainfall or irrigation. The inference is that in calibration of instruments for field use, the meters should be calibrated in a soil changing from a moist to a dry state.

RICHARDS and GARDNER (9) describe the underlying principles of indicating, recording, and differential tensiometers and mention the fact that temperature changes require a correction for capillary tension readings obtained with such instruments. In an earlier paper (8), RICHARDS discusses other factors influencing capillary potential and illustrates how the degree of packing of a soil affects the curvature of the water wedges between the soil particles, any change of which results in a different potential.

RICHARDS and NEAL (10) in a further study of tensiometers found considerably greater fluctuation in readings for shallow depths of 6 to 12 inches than at depths of 24 and 36 inches; this indicated a rapid response to rainfall and evaporation in the surface layer of soil. They also noted a diurnal fluctuation in capillary tension in the surface soil. This was explained on the basis of the varying evaporation rate of moisture from the soil and also by the possible effect of soil temperature and of the water in the tensiometer system.

ROGERS (11) devised a tensiometer that recorded capillary pulls of over 60 centimeters and incorporated an anti-freezing device that permitted the use of such instruments earlier in spring and later in fall than would ordi-

narily be possible. He gives calibration curves for several soils that indicate a striking difference, depending on texture.

Apparatus

Design of the Lake States moisture meter

Because of the necessity for getting more accurate control of soil moisture in forest nurseries in the Lake States, experiments were started in the spring of 1937, and carried out through 1938 and 1939, to determine the feasibility of various methods of determining soil moisture. Among the methods and devices tried were oven-drying, soil points, auto-irrigators, and tensiometers (**14**). The present paper will be confined to the presentation of data and experience obtained in constructing 42 tensiometers and from studies of several types of these instruments and their practical use in forest nurseries in growing drought-hardy and frost-resistant stock.

The first model (fig. 1) was patterned very closely after the instrument

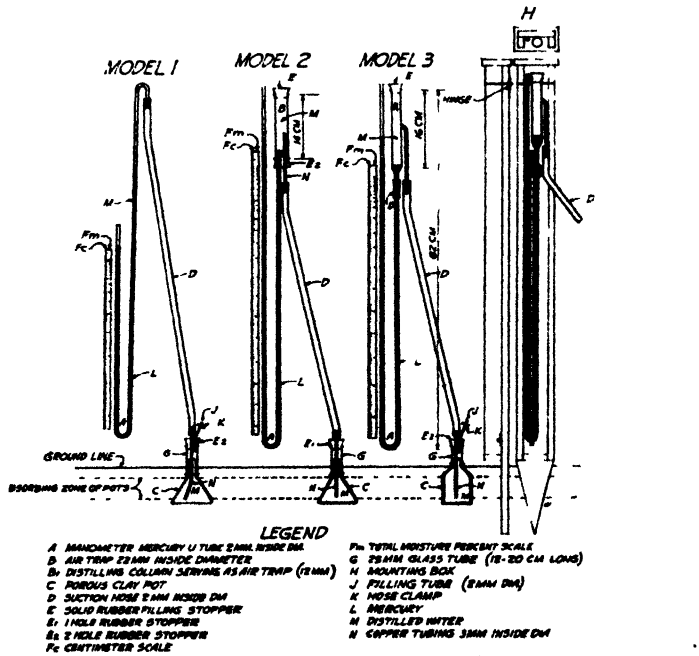


Fig. 1. Details of construction of several types of tensiometers.

designed by Heck (**3**). It differed mainly in that a Livingston auto-irrigator pot having a sloping porous side wall with an area of 100 cm.² was substituted for the elongated porous candle. A number of moisture meters of this type were made up, but did not prove entirely satisfactory owing to

the difficulty of preventing air from getting into the irrigator pot and eventually lodging in or near the top of the water tube connected with the mercury manometer. When a small amount of air lodged in the water column, the instrument did not function effectively.

One of the model 1 (fig. 1) tonometers, however, gave good results over a 2-month period in a bed of first-year red pine in the loamy fine sand soil at Hugo Sauer Nursery, Rhinelander, Wisconsin, in 1937. This instrument functioned successfully without addition of any water to the pot during the 2-month period; it must be admitted, however, that it was not subjected to a critical test because of favorable moisture conditions. The maximum mercury depression observed with this instrument was 30 centimeters.

Owing to the difficulty caused by air getting into the pot and water column, the instrument was redesigned and fitted with a device used for the dual purpose of adding water and trapping air. This device, designated as an air trap, consisted of a 25-mm. glass tube 14 cm. long (fig. 1, model 2) and was fitted at the top of the instrument. Both the mercury and water tubes were brought in at the bottom of the tube through a 2-hole rubber stopper carefully cemented into place to prevent any leakage of air. The water tube was carried up inside the air trap to a height of about 6 cm. Mercury was first placed in the U-tube in an amount sufficient to bring it to a height of about 0.5 cm. above the flat bottom of the air trap. Boiled distilled water was then run into the open upper end of the system in a very fine stream until the pot, suction hose, and air trap had been completely filled. A smooth-fitting rubber stopper (E) was then fitted tightly into the top of the air trap.

This design considerably simplified the addition of distilled water and the removal of air from the instrument. It also provided a storage reservoir for mercury and permitted the use of a shorter manometer than in moisture meters of the type illustrated by model 1, when due consideration is given to the effective length of the moisture percentage scale.

Variations in temperatures may affect the manometer readings. It thus appeared desirable to reduce the amount of water in the system above the ground line, and a smaller-bore air trap, indicated as item B_1 in model 3, was substituted. This piece had the further advantage that tight connections were more readily obtained. In the third model a larger porous clay jug of finer texture than the LIVINGSTON auto-irrigator pot was used. This Red Wing[3] pot was thick-walled (5 mm.) and had an absorbing surface of 180 $cm.^2$ on the vertical side wall, which was 24.5 cm. in circumference and 7.35 cm. high. The bottom and the sloping walls at the top of the cup were given a triple coat of waterproof varnish, so as to waterproof them and leave only the vertical wall as the porous element through which water moved.

[3] These consisted merely of unglazed Minnesota-Michigan souvenir jugs of "flower-pot clay" manufactured by the Red Wing Pottery Co., Red Wing, Minnesota.

This type of jug, in spite of its finer texture, gave more rapid reaction than did the LIVINGSTON cup and was less subject to entrance of air through the porous side walls. It was necessary, however, to test each individual pot by placing it in water and applying air pressure. Any pots with serious flaws, indicated by a stream of bubbles coming from the defective spot, were discarded. Pots with only minor flaws were made serviceable by putting a drop of DE KHOTINSKY cement on the leaky spot. Besides the vertical-walled Red Wing pot, several other types of porous cups, including "alundum" filters and spout tensiometer cups,[4] were tried. The latter type proved quite satisfactory but did not react as quickly as did the Red Wing jug because of the more limited absorbing surface. No doubt the smaller cup is an ideal type of cup for field conditions where fine-textured soils of undisturbed structure are being studied and where readings of over 40 cm. of mercury may be frequently encountered; for lighter soils adapted to growing of conifers, however, the Red Wing pot is quite satisfactory and costs only a fraction of the price charged for the more expensive clay cups or filters.

With the type of instrument shown in figure 1, model 3, any air in the system can readily be seen either in the glass neck (G) of the pot, or in the top of the air trap (B_1). To remove air it is necessary only to remove the stopper E from the air trap, release clamp K slightly, and pour distilled water into the top of the system until all air is removed. The clamp is reset and the stopper inserted slowly to prevent pushing mercury out of the high open end of the mercury manometer. After refilling with water, the mercury will gradually pull down till it is again in equilibrium. This will take from 1 to 20 hours, depending on how dry the soil is. The time required to attain equilibrium in a fairly dry soil can be shortened to a few hours, or less, by applying air pressure to the open end of the U-tube and forcing the mercury down to about the 60-cm. mark before filling the air trap with water and inserting the rubber stopper.

To prevent air leaks it is necessary to coat all joints with waterproof varnish before fitting them together and to treat them again after the first application has dried. Tight joints between the 25-mm. glass tube (G) and the porous pot were obtained by heating them gently in a Bunsen burner or alcohol blow torch and sealing them together with DE KHOTINSKY cement.

It should be mentioned here that placing of mercury in the model 2 and 3 tensiometers was easily accomplished by removing stopper E, running two pieces of thin copper wire down each side of the manometer, and pouring the mercury into the air trap, meanwhile jiggling the wires up and down.

[4] Used extensively by L. A. RICHARDS and manufactured by General Ceramics Co., Keasbey, N. J. These have an absorbing area 6.4 cm. high and are slightly tapered from 3.1 cm. diameter at top to 2.3 cm. diameter at bottom. The total absorbing area is about 57 cm.^{2}

When all traces of trapped air in the mercury had disappeared, the wires were removed and the system was then filled with water.

CALIBRATION OF THE MOISTURE METER

All moisture-measuring devices that work on the "suction pull" principle must be calibrated for each soil and generally for each depth, since the mercury pull obtained varies according to the texture of the soil particles. Fine soil particles such as silt and clay create a greater pull at a given moisture content than do coarser materials such as sands.

Two different methods of calibrating the moisture meters were tried. In one method the calibration was done directly in the nursery beds by setting up the instrument with a centimeter scale attached and with the porous cup so buried that the absorbing surface was at a depth between 3 inches and 6 inches. Soil samples were then taken in moisture cans over a period of 4 to 6 weeks by means of a smooth brass sampling tube one-half inch in diameter. Three or four cores were taken within a radius of one or two feet of the pot, with only the portion of the core from the 3- to 6-inch zone being retained for determination of the moisture content by oven-drying. All holes made by the sampling tube were filled with soil from the immediate vicinity, repacked with a wooden dowel, and the surface smoothed so as to avoid leaving either a depression or knoll. The depression of mercury in millimeters below the true equilibrium point[5] was recorded and the values obtained were plotted on coordinate paper and an averaging curve drawn. Then a scale that showed total moisture content and indicated the wilting coefficient[6] of the

[5] When water is contained in the system of the tensiometer shown in figure 1, the mercury in the open end of the manometer will drop from 5 to 7 cm. below the level of the mercury in the air trap. This is due to the hydrostatic head caused by the water in the air trap and in the rubber tube that connects the manometer with the porous pot C. The simplest way to determine the true zero or equilibrium point is to decide how far the center of the absorbing surface of the porous pot will be below the bottom of the mercury U tube in the actual field setup; then in the laboratory, set up the instrument with the porous cup the same distance below the bottom of the manometer and submerged in water to half the depth of the absorbing surface. In 10 to 20 minutes the mercury will come to the true zero point. This is marked on the box in which the manometer is housed and all readings during calibration are expressed in millimeters of mercury below that point.

[6] Determined indirectly by formula; wilting coefficient $= \frac{\text{moisture equivalent}}{1.84}$. For all practical purposes this value for sandy soils is so close to the actual wilting percentage that there is no reason for attempting to determine it by any other method. The value derived from $\frac{\text{moisture equivalent}}{2.0}$ appears to lie closer to the true wilting coefficient but it is felt that the difference in values derived by the two ratios would represent a certain desirable margin of safety in regulating moisture in forest nurseries. Therefore the first ratio mentioned is preferred.

soil at that location was substituted for the centimeter scale. The scale was made of aluminum embossing tape about one centimeter wide and 70 centimeters long. The figures and cross lines were drawn on the tape with India ink, allowed to dry, and then given several coats of waterproof varnish. The scale was held with tacks to the back of the wooden mounting box. The latter was painted white to reduce the solar heating of the mercury and water in the system, which has a tendency to cause errors in the readings. The portion of the moisture scale that was within 1 per cent. of the wilting coefficient was painted red to indicate a dangerously low moisture content.

The importance of degree of packing of soil and the necessity of undisturbed structure in calibration of tensiometers have been pointed out by several investigators, but in the case of forest nurseries the top 6 to 10 inches of soil have no definite structure because of frequent plowing, rototilling, and digging of trees. In using tensiometers in forest nurseries it is, however, considered important to obtain the correct degree of compaction of the soil around the porous cup. To attain this with maximum accuracy, it is advisable to obtain a sharp metal cylinder very slightly smaller in diameter than the vertical-walled porous cup and drive this into the soil between the seedling rows, remove the core, and then place the porous cup into the hole with a twisting motion. Soil can then be packed on top of the pot to level it off with the surface.

The field method of calibration outlined above has been found reasonably satisfactory but it has a disadvantage in that a calibration curve, for at least 90 per cent. of the range of moisture content in which plants will survive, may not be obtained for many months.

An alternate method of calibration is to obtain a jar of soil (from 0.5 to 1 gallon in volume) from the nursery area at which the tensiometer is to be set up and to do the calibrating in the laboratory. In the laboratory method of calibration the soil is thoroughly saturated, then packed around the porous cup. The top of the tin container is covered with a double layer of tight-fitting building paper, and the reading obtained next day, or as soon as it is evident that the moisture is evenly distributed and an equilibrium has been established. The cover is then removed and the entire tin with enclosed soil mass and porous cup is weighed. The soil is then allowed to dry out gradually, with readings being taken and weighings made morning and night until the soil appears quite dry and maximum mercury lifts of 30 to 60 cm. (depending on soil texture) have been recorded. The porous cup is then removed and the entire soil mass oven-dried at 105° C. The net oven dry weight of the soil is obtained and total moisture-content values for the entire range of the calibration are computed. This proved the most satisfactory method of calibration and results for any one soil could be obtained in a [illegible]- [illegible]-week period.

A second system of calibration in the laboratory was tried in which rather dry soil was packed around the porous cup in a large tin can, and the corresponding capillary tension recorded. Thirty to forty ml. of water were then added to the soil mass and a small portion of the soil was placed in a tight can for oven-drying. The process was repeated until a series of readings over a range of moisture contents had been obtained.

This method was discontinued because of the fact that changing volume-weight of the soil caused by repeated removal and repacking of the soil gave a rather wide dispersion of points for the average calibration curve. Moreover, since calibration is done on a rising moisture curve, the error incurred due to the hysteresis effect was correspondingly greater when the tensiometers were set up in the field, because, as pointed out by HAINES (4), the conditions in the field are generally represented by a falling moisture curve. This error for sandy soils usually amounts to 1 to 2 per cent. of moisture as read on the scale; for fine-textured soils it is considerably higher.

Experimental results

Figure 2 shows the calibration points and fitted curves obtained for six different soils. The physical properties of these soils are given in table I, where they are arranged in ascending order of texture. It will be noted that these appear in exactly the same order from left to right on figure 2. Of especial interest is the fact that coarse-textured material like soil no. 1, which is classified as a medium sand, exerts very little suction pull until it is within 3 or 4 per cent. of the computed wilting coefficient, and then increases very rapidly as it approaches the wilting coefficient. For the loamy sands and sandy loams the rise is somewhat more gradual, and for a silt loam it is even more uniform.

The maximum pull recorded in the calibration of the tensiometers was 54 centimeters. Beyond that point, air entered through the side walls of the porous pot and collected in sufficient volume in the system to make the readings unreliable. For forest nurseries located on sandy soils, however, a working range of 0 to 50 cm. of mercury pull is entirely adequate since it covers practically the entire range of moisture conditions encountered in nurseries in the periods of spring germination, active early summer growth, and hardening-off in late summer.

EFFECT OF ORGANIC MATTER ON CAPILLARY TENSION

In connection with the calibration of tensiometers, the rather practical question has come up as to whether or not such an instrument, once calibrated for a given spot in the nursery, could not be used year after year in that same location. Data on performance of such moisture meters over a period of two years indicate that this can be done safely, provided no heavy

TABLE I

PHYSICAL PROPERTIES OF SOILS USED IN MOISTURE-METER TESTS

SOIL AND CURVE NUMBER	NURSERY	INSTRUMENT NUMBER	MECHANICAL ANALYSIS									MOISTURE CONSTANTS		
			FINE GRAVEL 2.0–1.0 MM.	COARSE SAND 1.0–0.50 MM.	MEDIUM SAND 0.50–0.25 MM.	FINE SAND 0.25–0.10 MM.	VERY FINE SAND 0.10–0.05 MM.	TOTAL SILT + CLAY. BELOW 0.05 MM.	FINE MATERIAL SEPARATES: SILT 0.05 TO 0.005 MM.	CLAY 0.005 TO 0.002 MM.	FINE CLAY. UNDER 0.002 MM.	MOISTURE EQUIVALENT*	WILTING COEFFICIENT†	HYGROSCOPIC COEFFICIENT‡
			%	%	%	%	%	%	%	%	%	%	%	%
1	Hayward, Wis.	39	4.1	21.7	37.1	22.8	4.9	9.4	5.0	0.8	3.6	4.0	2.2	1.5
2	Wellston, Mich.	38	0.8	8.1	40.1	35.0	3.8	12.2	6.8	1.4	4.0	5.7	3.1	2.1
3	Wellston, Mich.	37	1.0	8.9	39.2	35.2	2.4	13.3	6.9	1.3	5.1	7.8	4.2	2.9
4	Butternut, Wis.	41	0.8	2.0	17.7	50.0	14.2	15.3	9.3	0.9	5.1	8.5	4.6	3.1
5	Knife River, Minn.	35	10.8	15.9	18.0	21.3	7.2	26.8	14.9	1.3	10.6	14.2	7.7	5.2
6	Licking, Mo.	36	1.1	2.5	9.8	17.9	3.1	65.6	42.4	8.4	14.8	19.1	10.4	7.0

* Represents percentage of moisture held against a force equal to 1,000 times force of gravity, and determined by standard centrifuging method. Determined by Division of Soils, University of Minnesota.

† Computed from formula: Wilting coefficient $= \frac{\text{moisture equivalent}}{1.84}$. This value represents point at or near which temporary wilting occurs on most conifers growing under nursery conditions. Plants will recover if watered shortly after wilting occurs.

‡ Computed from formula: Hygroscopic coefficient $= \frac{\text{moisture equivalent}}{2.71}$. This value represents point at which all trees die if exposed for any appreciable length of time. Serious mortality often occurs, even before moisture drops to the hygroscopic coefficient.

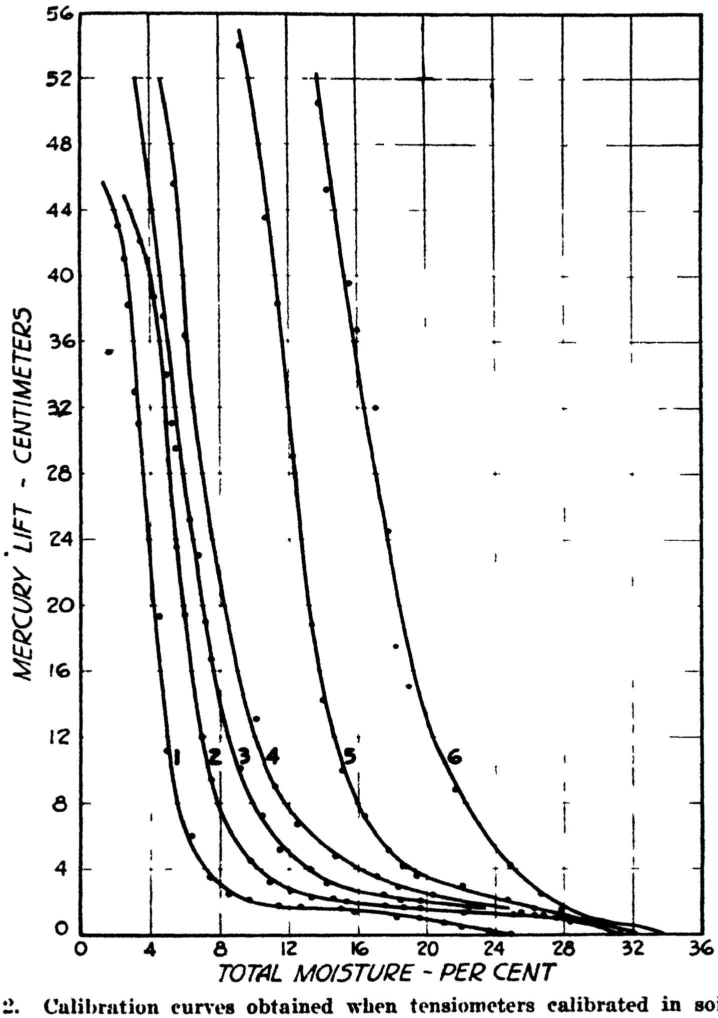


FIG. 2. Calibration curves obtained when tensiometers calibrated in soils ranging from sand (left) to silt loam (right). A Red Wing pot was used as the porous element.

addition of organic matter is made such as is provided by peat, compost, animal manures, and possibly soiling crops. Some error, however, will occur in the accuracy of the instruments because the settling effect of irrigation and rain gradually causes a compaction of the seedbed, which at the time of sowing is in a loosely packed condition. This change in volume-weight as the season progresses necessarily causes a slight error in readings of the total moisture percentage.

The soil-maintenance program in the Federal and State forest nurseries in the Lake States requires the addition of rather large quantities of peat and

compost after removal of a crop of trees. The addition of such material will cause a marked difference in the type of calibration curve obtained for sandy soils, as is shown in figure 3.

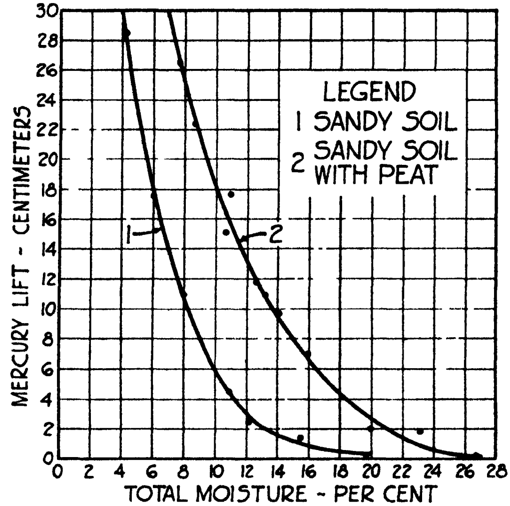


FIG. 3. Effect of peat on calibration curve of a sandy nursery soil containing 12 per cent. of silt plus clay. The soil for curve no. 2 was the same as used for curve no. 1, except that it contained 1 pound of peat (oven-dry basis) for every 20 pounds of soil, a treatment equivalent to about 50 tons per acre of dry peat plowed into the top 6 inches of soil.

In this case the addition of 50 tons per acre of acid peat (oven dry-weight basis) obtained from a spruce-tamarack swamp changed the calibration curve from that of a rather typical fine sand to the type of curve ordinarily obtained for a light sandy loam. The explanation for this is quite simple. The addition of a large quantity of organic matter increases the number of contact points and the pore space. As a consequence, in a soil of equal total moisture content, it decreases the radius of curvature of the wedges of water that occur between the particles of soil. This results in an increase of the capillary tension of the soil if held at the same total moisture per cent.

Discussion

Interpretation of calibration curves

From the data obtained from the calibration curve it is an easy matter to make a scale for the tensiometer showing total moisture content and the wilting coefficient. These scales are attached to the tensiometers in place of the centimeter scale, and thereafter the nurseryman can readily compute the moisture content of that portion of the nursery by reading the total

moisture content and subtracting from it the indicated wilting coefficient to obtain the percentage of available[7] moisture in the soil.

As an aid to nurserymen in judging relative soil-moisture in its relation to active growth, table II incorporates tensiometer information for the better sands and loamy sand nursery soils which are the usual textures in most Federal and State nurseries found in the Lake States. The data are based on observations with tensiometers at Rhinelander and Hayward, Wisconsin, and Manistique, Michigan. Similar tables can be made up for the sandier as well as the finer-textured soils, but these would require some adjustment in the second and third columns, especially for the fine-textured soils. Refinements are necessary for individual species and are yet to be worked out.

It will be noted that when the mercury depression exceeds 35 centimeters, the moisture content for the loamy sand soils is at, or within, a few per cent. of the wilting coefficient; watering will thus be necessary soon after this pull is attained. When the tension is between 25 and 35 cm., growth (especially of tops) is slow, and between 15 and 25 it is fair to good. Growth is best when the suction pull is between 2 and 15 cm. When the capillary tension is less than about 2 cm., the soil is definitely soggy and sometimes even water-logged. Fortunately, in the sandy soils of most Lake States nurseries an excess of water due to heavy rains is soon lost by underdrainage, and a saturated condition does not occur for more than a few hours.

The correlation of the type of calibration curve obtained with the texture and moisture equivalent of soils suggests the possibility of dispensing with the calibration of the moisture meters. On the basis of a mechanical analysis of the soil and a determination of the moisture equivalent, it might be feasible to make up a scale showing relative soil-moisture status of the soil, using the terms similar to those given in column 1 of table II. This would, however, require access to a chart showing calibration curves of at least 30 to 50 soils having a range of silt-plus-clay content from 2 per cent. to 90 per cent. and would, furthermore, be contingent on complete information on effect of packing, structure, organic matter, soil nutrients, and volume weight of the soil and their effect on tensiometer readings under field conditions. For the present, the safest procedure will necessarily rely on a calibration of each instrument for every field setup since the individual and composite effect of the above factors has not been determined to any degree of refinement.

[7] "Available" water as expressed here refers to the percentage of moisture above the computed wilting coefficient. In reality some of the water between the computed hygroscopic and wilting coefficient values can be extracted by conifers but this moisture, is of no consequence in producing any appreciable growth, as it is barely adequate to sustain life for a comparatively short time in the mesophytic type of plants. This is especially true for the species of pine and spruce commonly grown in nurseries in Michigan, Wisconsin and Minnesota.

TABLE II

CORRELATION OF TENSIOMETER READINGS WITH MOISTURE CONTENT AND GROWING CONDITIONS IN LOAMY SAND SOIL USED FOR PRODUCTION OF CONIFERS

SOIL MOISTURE STATUS	MERCURY PULL	APPROXIMATE AVAILABLE MOISTURE CONTENT	REMARKS
	cm.	%	
Very dry	Over 35	0–2	Conifers are at or near wilting coefficient. Serious mortality may occur if water is not applied. Moisture should not be held in this range for prolonged periods during active growth period
Dry	25–35	2–4	Use this range principally in hardening stock
Fresh	15–25	4–6	Allow moisture to reach this range, or slightly lower, even during active growing season, to induce drought hardiness
Moist	2–15	6–14	Active top and root growth occurs
Wet	Under 2	Over 14	Too moist. Do not maintain moisture in this range for any protracted length of time in conifer nurseries

Experience, backed by experimental evidence, indicates that in nursery practice the moisture content of the top eight inches of soil should be maintained mostly in the "fresh" and in the drier part of the "moist" range. This 8-inch level is specified because in most Lake States nurseries usually from 70 to 95 per cent. of the root system of seedling and transplant conifers is found in the upper eight inches. Consequently, in using tensiometers in conifer nurseries, the porous cups are usually set so that the absorbing surface is somewhere in the zone indicated. For shallow-rooted or first-year seedlings, the cup is generally placed at the 1- to 4-inch zone, while for stock 2 years of age or older, the cup is set at the 2- to 5-inch or 3- to 6-inch zone.

In the latter part of the growing season, which in the Lake States means August 15 to September 15, the moisture content should be held, if conditions permit, in the "dry" and "fresh" range, in order to harden the stock properly. The hardening process decreases moisture content of the stock, reduces rate of top growth and leaf surface, and accelerates the root development of the plant. Thus a comparatively low top-root ratio results and the well-balanced plants so produced have a better chance of surviving drought and winter killing. A comparatively low soil moisture content in conifer beds in late summer will also hasten dormancy and increase frost resistance.

Performance of tensiometers in forest nurseries

Figures 4 and 5 give the records of performance of two tensiometers in forest nurseries, one at Rhinelander, Wisconsin, and the other at Manistique,

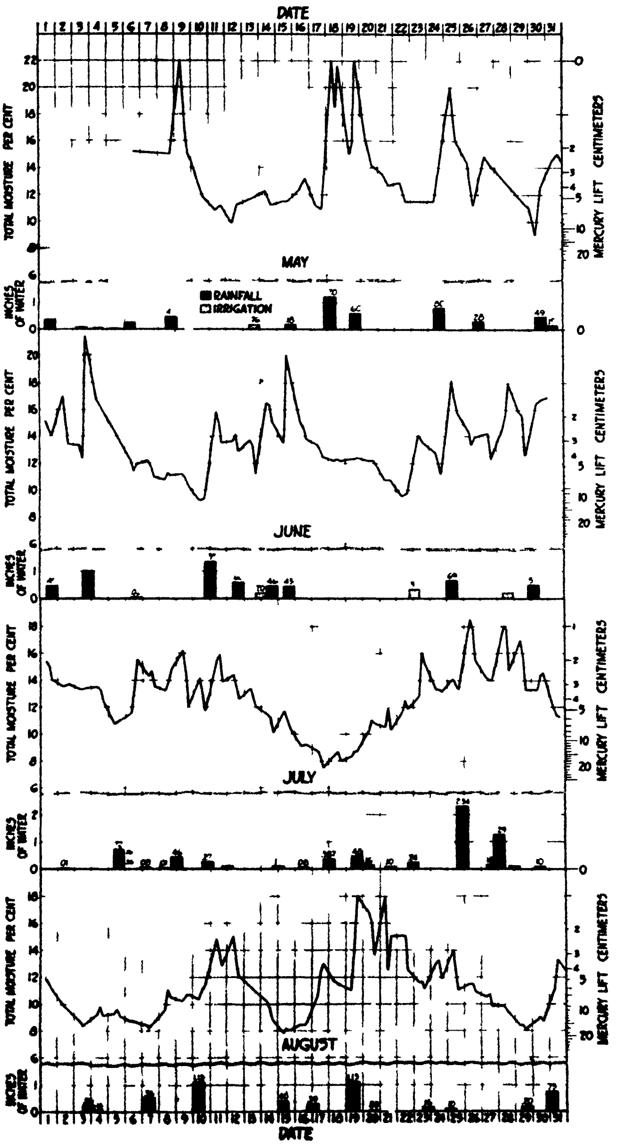


Fig. 4. Soil moisture content at a depth of 4 inches of a loamy fine sand soil as determined by a tensiometer in an experimental bed of 2–0 jack pine, Hugo Sauer Nursery, Rhinelander, Wisconsin, 1938. The soil had a silt plus clay content of 15 per cent., a moisture equivalent of 8 per cent., and a computed wilting coefficient of 4.3 per cent.

Michigan.[8] Readings were generally taken at least once, and often twice daily. The soils in these nurseries are a loamy fine sand and a fine sand respectively.

The correlation between tensiometer readings and the frequency and amount of precipitation and irrigation water received is noteworthy. It will also be noted that each of the soils had a fairly definite "tapering off" zone in the moisture curve, which is about 14 per cent. and 7 per cent., respectively, for Rhinelander and Manistique nurseries. This value for both soils lies between 1.50 and 1.75 times the moisture equivalent value. In other words, each soil has a certain field holding capacity and any water applied in excess of this amount drains away very rapidly into the subsoil. This rapid drop of soil moisture content after the soil has received a thorough soaking by precipitation or irrigation is strikingly illustrated in figure 5 and is brought out in more detail in figure 6, which shows a drop from 23 to 15 per cent. in total moisture content in the brief period of 6 hours, largely because of loss by underdrainage. There was apparently very little water loss by evaporation or transpiration from 5 P.M. of one day to 8 A.M. of the next, but after 9 A.M. on the second day these forces again became active in causing water loss.

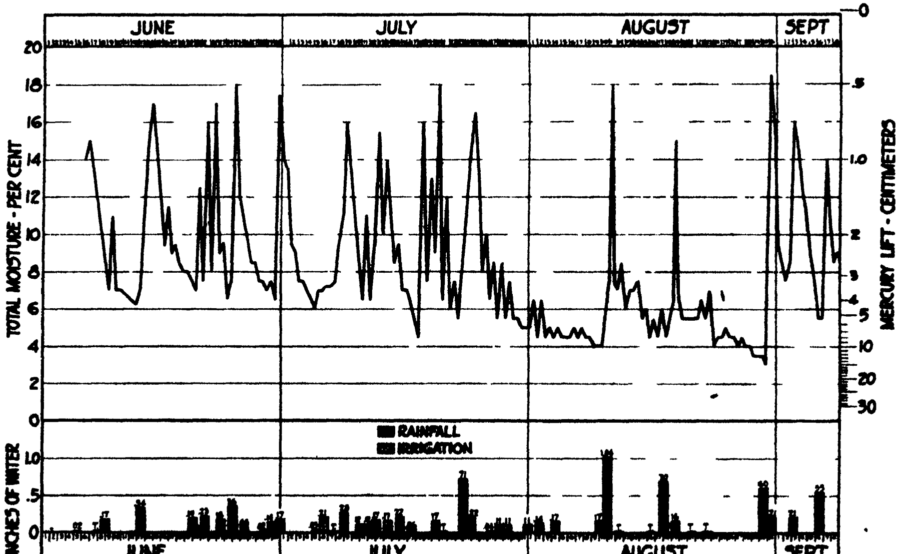


FIG. 5. Soil moisture content at a depth of 3 inches of a fine sand soil as determined by a tensiometer in a bed of 2–0 red pine, Wyman Nursery, Manistique, Michigan, 1938. The soil had a silt-plus-clay content of 1.8 per cent., a moisture equivalent of 3.8 per cent., and a wilting coefficient of 2.1 per cent.

[8] The record for Manistique was furnished through the cooperation of Messrs. E. F. BIEBESHEIMER and FLOYD STRUBLE, Nursery Superintendent and Assistant Superintendent respectively.

VALUE OF TENSIOMETERS

The tensiometers have practical value to the nurseryman. He can control water supply and thus provide drought-hardy planting stock and prevent wastage of water by unnecessary irrigation and subsequent loss of highly soluble fertilizers, such as ammonium sulphate and potash. Even though the soluble fertilizers are composted and partially adsorbed with peat, muck, or other organic materials, some loss is certain to occur and it will be accelerated by excessive watering.

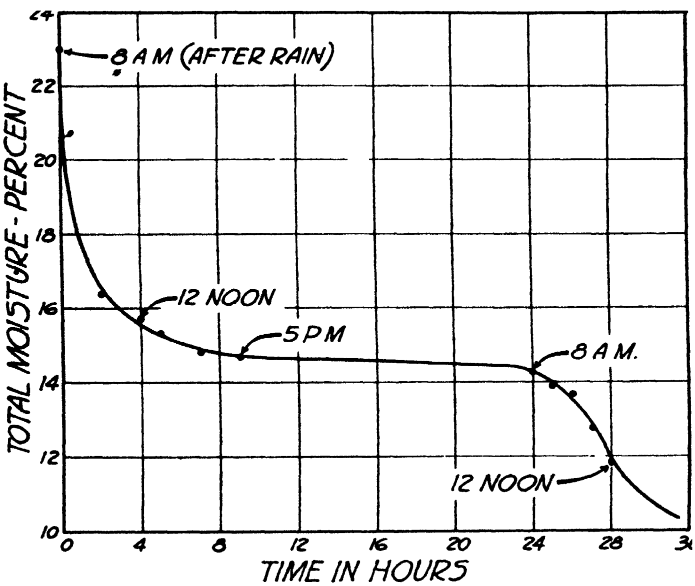


FIG. 6. Graph illustrating loss of moisture in a loamy fine sand soil, Hugo Sauer Nursery, Rhinelander, Wisconsin, August 3 and 4, 1937. Porous cup of tensiometer was 2 inches below surface of bed of 1–0 jack pine.

The tensiometers are equally valuable in periods of severe drought; they indicate depth of penetration of irrigation water, especially if the porous cups are set at several depths in the rooting zone of the seed bed. Thus they indicate the effectiveness of the water applied and furnish warning of impending injury or actual death of nursery stock because of low soil moisture content. Such a drought occurred in 1936 in the Lake States. High temperatures combined with markedly subnormal rainfall resulted in rather sudden losses in nurseries, especially in the center of the second-year seedbeds of pine and spruce. A very low soil moisture content is not, of course, conducive to the best growth of conifers. It is necessary to strike a happy medium between the two extremes mentioned.

It is obvious from the records obtained in this study that tensiometers

can be of valuable assistance in understanding fully the rôle of moisture in forest nurseries or in experimental plots where the moisture content of the soil is being studied in relation to drought hardiness, growth, and yield. To obtain a similar detailed record with the usual method of soil sampling and oven drying would require a large amount of work. Moreover, in some experimental work it would be very undesirable to disturb the plots by removing several hundred soil samples over a period of three or four months.

Instruments based on the tensiometer principle will, no doubt, be of increasing value in the field of plant physiology, floriculture, horticulture, agronomy, ecology, and hydrology. There is a definite need for commercial development of such an instrument, which would combine the best features of the tensiometers built by various investigators.[9]

Summary

A description is given of several types of tensiometers and of their use in gauging the necessity of watering in conifer nurseries in the Lake States. Records are presented showing the performance of two of the instruments for a period of three to four months in two different nurseries. Calibration technique is described and the capillary potential of soil is shown to vary by texture and by addition of organic matter. A discussion is given to show how watering may influence development of nursery stock, especially as regards size, top-root ratio, frost hardiness, and drought resistance.

Practical values of this type of "moisture meter" to the nurseryman include: (1) prevention of injury or death of trees due to the warning given of the presence of low soil moisture content in periods of heat and droughts; (2) aid in the regulation of moisture, especially during the "hardening-off" period, in such a manner as to obtain planting stock that is frost resistant and drought hardy; (3) the prevention of excessive watering with concomitant expenses and the subsequent loss, by underdrainage, of highly soluble commercial fertilizers.

Lake States Forest Experiment Station
University Farm
St. Paul, Minnesota

LITERATURE CITED

1. Bouyoucos, G. J. Evaporating the water with burning alcohol as a rapid means of determining moisture content of soils. Soil Sci. **44**: 377–383. 1937.
2. Heath, O. V. S. A method of water control for sand cultures. Ann. Bot. **43**: 71–79. 1929.
3. Heck, A. F. A soil hygrometer for irrigated cane lands of Hawaii. Jour. Amer. Soc. Agron. **25**: 272–278. 1934.

[9] Instruments of this type are already being manufactured commercially in England.

4. HAINES, W. B. Studies in the physical properties of soil: V. The hysteresis effect in capillary properties and the modes of moisture distribution associated therewith. Jour. Agr. Sci. **20**: 97–116. 1930.

5. KORNEV, V. G. The suction force of soils. Zhurnal Opitnoi Agron. (Russian Jour. Exp. Agron.) **22**: 105–111. (1921–23). 1924. Abstract in Soil Sci. **17**: 428. 1924.

6. LIVINGSTON, BURTON E., and NOREM, W. LUTHER. The water-supplying power and water-absorbing power of soil as related to wilting of wheat and Coleus in greenhouse pot cultures. Soil Sci. **43**: 177–204. 1937.

7. LYNDE, C. J., and DUPRÉ, H. A. On a new method of measuring the capillary lift of soils. Jour. Amer. Soc. Agron. **5**: 107–116. 1913.

8. RICHARDS, L. A. The usefulness of capillary potential to soil moisture and plant investigators. Jour. Agr. Res. **37**: 719–742. 1928.

9. ————, and GARDNER, WILLARD. Tensiometers for measuring the capillary tension of soil water. Jour. Amer. Soc. Agron. **28**: 352–358. 1936.

10. ————, and NEAL O. R. Some field observations with tensiometers. Proc. Soil Sci. Soc. Amer. **1**: 71–91. 1936.

11. ROGERS, W. S. A soil moisture meter depending on the capillary pull of the soil. Jour. Agr. Sci. **25**: 326–343. 1933.

12. SHIRLEY, H. L. A method of studying drought resistance in plants. Science n.s. **79**: 14–16. 1934.

13. SMITH, W. O. Sorption in an ideal soil. Soil Sci. **41**: 209–230. 1936.

14. STOECKELER, J. H. Measuring soil moisture content in the forest nursery. Preliminary statement issued for the use of field men. Lake States Forest Experiment Station. Mimeographed. July 30, 1937.

THE EFFECT OF VARIOUS WAVEBANDS OF SUPPLEMENTARY RADIATION ON THE PHOTOPERIODIC RESPONSE OF CERTAIN PLANTS[1]

R. B. WITHROW AND ALICE P. WITHROW

(WITH SIX FIGURES)

Introduction

The effect of various wavebands of visible radiation, used to lengthen short daylight periods, on the photoperiodic response of plants has been the subject of considerable disagreement. SCHAPPELLE (**9**) concludes that supplemental red and blue radiation are equally effective in inducing flowering in radish and spinach, that red is more effective than blue in the case of China aster and early flowering cosmos and that blue is more effective than red in the case of lettuce, although red induces flowering. Salvia, chrysanthemum, Kalanchoe, teosinte, and Maryland Broadleaf tobacco failed to flower under any radiation prolonging the daylength to more than ten hours.

FUNKE (**3, 4, 5**) divided his results into four types of flowering response when he supplemented an 8-hour day with red, blue, and white radiations. Group I included those plants which flowered under short days and blue at the same time and under white and red at the same time; plants of group II failed to flower under short days but flowered under all long day conditions; those in group III flowered only under white radiation and under no other condition; and those in group IV flowered simultaneously under white and blue radiation and under red and dark at the same time. He used a light intensity of from 30 to 60 lux (approximately 3 to 6 foot candles) and a temperature of 12° to 15° C.

RASUMOV (**8**) found that, in general, red light acted like white light in inducing flowering in long day plants and inhibiting flowering in short day plants; green, blue, and violet light acted like darkness. He found, however, a variable sensitivity to differences in wavelengths of radiation.

WITHROW and BENEDICT (**11**), and WITHROW and BIEBEL (**12**) found that red and white radiation induced early flowering in long day plants and inhibited flowering in short day plants. Short day plants flowered in days lengthened with green and blue light, as well as under short days. Some long day plants, such as China aster and *Helianthus cucumerifolius* also flowered under blue radiation at the same time as under the red, while Scabiosa, another long day plant, failed to flower under the blue. The plants were grown at 50° F. night and 55° F. day temperature.

[1] Contribution from the Department of Horticulture, Indiana Agricultural Experiment Station, Lafayette, Indiana.

These results appear to disagree chiefly on the effect of the blue radiation. Most of the workers agree that red radiation is effective in inhibiting flowering in short day plants and inducing flowering in long day plants.

Considering that the flowering responses of many long and short day plants are strongly influenced by very low intensities of incandescent lamp radiation (**1**, **11**) applied to prolong short winter days, it is to be expected that the more sensitive plants would be affected by very small amounts of red light transmitted by the blue filters and by unfiltered radiation scattered from the radiation equipment. It has been shown (**11**) that only a few hundredths of a foot candle are sufficient to induce the flowering of China aster and intensities above 0.5 foot candle inhibits flowering in Biloxi soybean (**1**). Unless very special precautions are taken in the design of radiation equipment, extraneous red radiation may mask the effect of the blue band transmitted by the filter system and the result might apparently indicate a second maximum in the spectral response curve of the most sensitive types of plants. Less sensitive plants would probably not be appreciably influenced by these extraneous radiations and a single maximum in the red would occur.

The present investigation, therefore, is an attempt to determine the relative effect of supplemental blue radiation on the flowering response of certain long and short day plants and to further corroborate results secured with the longer wavelengths of visible radiation.

Procedure

The plants were grown in a greenhouse in an automatic sub-irrigation gravel culture system (**13**) with the nutrient solution shown in table I. The

TABLE I

NUTRIENT SOLUTION FORMULA

SALT	CONCENTRATION-MILLI-MOLAR
$MgSO_4$	1
$Ca(H_2PO_4)_2$	1
KNO_3	10
K_2SO_4	5
$CaSO_4$	8

solution was pumped into the beds on 4-hour cycles. The pH was maintained between 5.5 and 6.5. The temperature was manually controlled at approximately 62–65° F. day and 52–55° F. night.

Callistephus chinensis, variety Heart of France (China aster), *Scabiosa atropurpurea*, variety Azure Fairy, *Spinacia oleracea*, variety Nobel (spinach), *Soja max*, variety Mukden (soybean), *Xanthium pennsylvanicum*, *Salvia splendens*, variety Fairly Tall, and *Tithonia speciosa* were the plant materials used in the experiment. All seed except soybean were sown

in sand flats. The sand flats of long day plants were placed immediately after seeding under short day conditions; those of short day plants were placed under a 24-hour day of daylight supplemented with 20 foot candles of radiation from an incandescent tungsten filament lamp in order to prevent induction of floral buds. The seedlings were transplanted into the radiation plots in the gravel benches as soon as they were large enough. Soybean was sown directly into the plots of gravel.

Five radiation conditions were included in the investigation. The short day plot received daylengths as given in table II. The other four plots received red, yellow-green, and blue radiation of approximately 100 ergs/cm.^{2}/second, and blue of 400 ergs/cm.^{2}/second to supplement the natural daylength to a 24-hour day. No dark period was given the plants in the radiation plots. The lamp and filter equipment, together with transmission data, are presented in table III and figure 1. For the blue, the two most intense blue mercury lines at 4047Å and 4358Å were used and for the yellow-green, the green line at 5461Å, and the yellow doublet at 5770Å and 5791Å. The radiation was measured by means of a thermopile calibrated against a standard lamp of the U. S. Bureau of Standards.

TABLE II

DAYLENGTH FROM OCTOBER 1 TO MAY 1, LAYFAYETTE, INDIANA

DATE	DAYLENGTH	
	hr.	*min.*
Oct. 1	11	46
Oct. 15	11	10
Nov. 1	10	28
Nov. 15	9	57
Dec. 1	9	30
Dec. 15	9	18
Dec. 21	9	16
Jan. 1	9	20
Jan. 15	9	36
Feb. 1	10	9
Feb. 15	10	41
Mar. 1	11	17
Mar. 15	11	54
Apr. 1	12	40
Apr. 15	13	16
May 1	13	56

The gelatin filters consisted of dyed gelatin poured onto panes of 20-inch square window glass and placed in a light-tight rack soldered to the bottom of the primary filter cells. Each primary filter cell consisted of a sheet-iron tank 6 inches deep and 22 inches square, having an open bottom into which a pane of glass was sealed with asphalt. The interior of the tank was painted with asphalt to protect it from the corrosive action of acidulated copper sul-

TABLE III

DATA ON RADIATION EQUIPMENT

BAND AND INTENSITY	WAVELENGTH LIMITS	LIGHT SOURCE	PRIMARY FILTER; AQUEOUS	SECONDARY FILTER OF DYED GELATIN
ergs/cm.²/sec.	Å			
Blue 100	4047 and 4358 Hg lines	250-watt type H_2, high pressure Hg arc	10 cm. 2 per cent. $CuSO_4 \cdot 5H_2O$	Victoria pure blue BO and crystal violet
Blue 4X 400	4047 and 4358 Hg lines	400-watt type H_1, high pressure Hg arc	10 cm. 2 per cent. $CuSO_4 \cdot 5H_2O$	Victoria pure blue BO and crystal violet
Yellow-green 100	5461, 5770 and 5791 Hg lines	400-watt type H_1, high pressure Hg arc	10 cm. 4 per cent. $CuSO_4 \cdot 5H_2O$	Pontamine fast yellow 5GL and orange G
Red 100	6400 to approximately 9000. Continuous spectrum	500-watt gas filled tungsten filament lamp	10 cm. 1 per cent. $CuSO_4 \cdot 5H_2O$	Chrysoidine Y and crystal violet

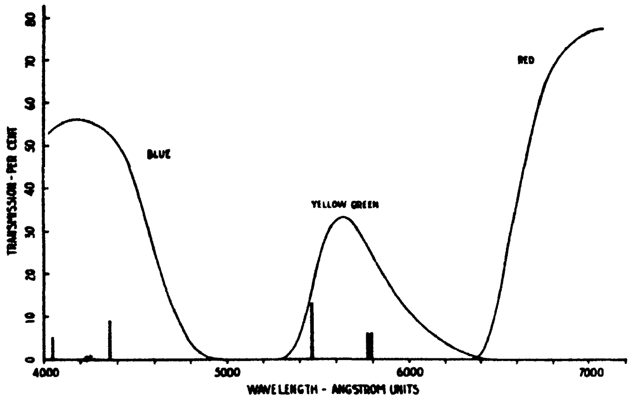


FIG. 1. Percentage transmission of filter systems.

phate solutions. The top of the filter cell was covered with a lid having a circular opening 17 inches in diameter upon which rested the 18-inch diameter porcelain enamelled low bay type of reflector containing the appropriate lamp. It was possible to eliminate practically all extraneous light with this equipment.

The individual experimental plots were separated from each other with opaque black rubberized cloth. The leaf areas were measured with a photoelectric surface type photometer having a 12-inch aperture (**10**). The plant material was harvested, weighed and dried at 100° C. in a forced draft thermostatically controlled drying oven for about 14 hours.

Results

Data are presented on the date of appearance of macroscopic flower buds, date of flower opening, on height and weight and, in the case of aster and soybean, on leaf number and area. These data are given in tables IV through X. Photographs of the plant material are shown in figures 2, 3, 4, 5, and 6. The height, weight and leaf area data were taken from 50 to 70 days after seeding, depending on the germination and growth rate of the plant. Flowering data on aster were taken into the spring months when the natural daylength conditions were long enough to induce flowering in aster without the addition of artificial light.

All the long day plants set bud under red and yellow-green light. Scabiosa and spinach failed to set bud under blue light or the short day condition. The short day and blue plots of aster were continued into the spring months when the daylength was around 12 hours. Under these daylengths, aster showed a slight decrease in budding time under blue radiation, a greater decrease occurring under the higher intensity blue, as compared to those plants grown in natural daylengths.

TABLE IV

FLOWERING AND WEIGHT DATA ON *Callistephus chinensis*, VARIETY HEART OF FRANCE
SEEDING DATE: OCTOBER 27, 1938
IRRADIATION DATES: DECEMBER 12, 1938, TO HARVEST
HARVEST DATE: JANUARY 13, 1939
NUMBER OF PLANTS PER TREATMENT: 40 FOR WEIGHT, AND 20 FOR FLOWERING DATA

TREATMENT	NO. DAYS TO BUD*	NO. DAYS TO FLOWER*	AV. BUDS AND FLOWERS	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO	AV. NO. LEAVES	AV. LEAF AREA/LEAF	AV. LEAF AREA/PLANT
					TOP	ROOT	TOTAL					
				cm.	*mg.*	*mg.*	*mg.*				*cm.*^{2}	*cm.*^{2}
Short day	102	140	47	0.3	170	63	233	8.4	2.7	16	3.7	60
Blue	94	135	38	0.3	222	70	292	8.9	3.1	16	4.6	74
Blue 4X	90	123	26	0.5	203	51	254	8.2	4.0	12	6.0	75
Yellow-green	58	107	10	0.5	221	48	269	7.7	4.6	11	7.6	84
Red	58	101	8	4.9	266	54	320	7.8	4.9	11	9.4	103

* From beginning of long day treatment.

TABLE V

FLOWERING AND WEIGHT DATA ON *Scabiosa atropurpurea*, VARIETY AZURE FAIRY

SEEDING DATE: OCTOBER 7, 1938
IRRADIATION DATES: DECEMBER 2, 1938, TO HARVEST
HARVEST DATE: JANUARY 1, 1939
NUMBER OF PLANTS PER TREATMENT: 40 FOR WEIGHT AND 20 FOR FLOWERING DATA

TREATMENT	NO. DAYS TO BUD*	NO. DAYS TO FLOWER*	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day			2.9	471	111	582	7.4	4.2
Blue			3.4	555	145	700	7.3	3.8
Blue 4X			2.9	475	116	591	7.0	4.1
Yellow-green	110		4.0	478	108	586	7.1	4.4
Red	61	99	14.5	545	101	646	7.5	5.4

* From beginning of long day treatment.

The short day plants all failed to form macroscopic flower buds under red and yellow-green radiation and all set bud under both intensities of blue light and under the short day treatment. The low intensity blue affected the budding time either not at all or increased it slightly. The higher intensity delayed the budding time from 20 to 50 per cent. in all the short day plants with the exception of Salvia.

With aster, Scabiosa, Xanthium and soybean, all the radiation treatments increased the total dry weight over that of plants in the short day. Spinach

TABLE VI

FLOWERING AND WEIGHT DATA ON *Spinacia oleracea*, VARIETY NOBEL

SEEDING DATE: OCTOBER 27, 1938
IRRADIATION DATES: DECEMBER 13, 1938, TO HARVEST
HARVEST DATE: JANUARY 20, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	NO. DAYS TO BUD*	NO. DAYS TO FLOWER*	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day			0.4	505	77	582	9.3	6.4
Blue			0.5	478	66	544	9.6	7.2
Blue 4X			0.6	457	63	520	8.3	7.3
Yellow-green	20	34	27.4	872	113	985	7.8	7.8
Red	20	34	35.8	870	104	974	8.2	8.4

* From beginning of long day treatment.

TABLE VII

FLOWERING AND WEIGHT DATA ON *Soja max*, VARIETY MUKDEN
SEEDING DATE: DECEMBER 10, 1938
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: FEBRUARY 3, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	NO. DAYS TO BUD	NO. DAYS TO FLOWER	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO	AV. NO. LEAVES	AV. LEAF AREA/PLANT	AV. LEAF AREA/LEAF
				TOP	ROOT	TOTAL					
			cm.	*mg.*	*mg.*	*mg.*	%			*cm.*^{2}	*cm.*^{2}
Short day	41	85	13.3	546	56	602	15.1	9.7	5.6	16.1	90
Blue	43	87	19.5	676	63	739	16.1	10.7	6.1	19.7	120
Blue 4X	62	100	21.9	564	52	616	13.7	10.8	5.2	21.6	112
Yellow-green			43.6	805	75	880	11.7	10.7	6.2	22.5	140
Red			45.2	600	45	645	11.8	13.4	5.7	23.2	131

TABLE VIII

FLOWERING AND WEIGHT DATA ON *Xanthium pennsylvanicum*
SEEDING DATE: JANUARY 5, 1939
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: MARCH 17, 1939
NUMBER OF PLANTS PER TREATMENT: 10

TREATMENT	NO. DAYS TO BUD	NO. DAYS TO FLOWER	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO
				TOP	ROOT	TOTAL		
			cm.	*mg.*	*mg.*	*mg.*	%	
Short day	33	57	24.8	3720	590	4310	12.2	6.3
Blue	33	57	32.0	3680	610	4290	13.2	6.0
Blue 4X	47	71	35.5	3100	610	3710	11.1	5.1
Yellow-green			40.3	3670	690	4360	11.1	5.3
Red			43.3	3690	720	4410	11.5	5.1

TABLE IX

FLOWERING AND WEIGHT DATA ON *Xanthium pennsylvanicum*
SEEDING DATE: JUNE 26, 1939
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: JULY 15, 1939
NUMBER OF PLANTS PER TREATMENT: 30

TREATMENT	NO. DAYS TO BUD	AV. HEIGHT	AV. DRY WEIGHT			DRY MATTER	TOP/ROOT RATIO
			TOP	ROOT	TOTAL		
		cm.	*mg.*	*mg.*	*mg.*	%	
9 hour day	14	7.3	355	46	401	9.0	7.7
24 hour day		9.6	289	41	330	9.0	7.2

TABLE X

FLOWERING DATA ON *Salvia splendens*, VARIETY FAIRLY TALL
SEEDING DATE: JANUARY 3, 1939
IRRADIATION DATE: GERMINATION TO HARVEST
HARVEST DATE: APRIL 21, 1939
NUMBER OF PLANTS PER TREATMENT: 12

TREATMENT	AV. HEIGHT	NO. DAYS TO BUD	NO. DAYS TO FLOWER
	cm.		
Short day	111*	74	98
Blue	113*	74	104
Blue 4X	114*	76	108
Yellow-green	121		
Red	130		

* Height to base of flowering spike.

TABLE XI

FLOWERING DATA ON *Tithonia speciosa*
SEEDING DATE: DECEMBER 5, 1938
IRRADIATION DATES: GERMINATION TO HARVEST
HARVEST DATE: MARCH 21, 1939
NUMBER OF PLANTS PER TREATMENT: 20

TREATMENT	NO. DAYS TO BUD	NO. DAYS TO FLOWER	AV. NUMBER OF FLOWERS AND BUDS
Short day	38	74	32 flowers and flower buds
Blue	51	78	28 flowers and flower buds
Blue 4X	56	86	21 flowers and flower buds
Yellow-green			15 vegetative buds
Red			18 vegetative buds

increased in dry matter only under red and yellow-green radiation and the most pronounced increases generally occurred under the influence of the longer wavelengths. The longer wavelengths caused a decrease in the percentage of dry matter in all the plants studied except Scabiosa, where there was little variation between any of the plots at the time of harvest. There was a definite decrease in the percentage of dry matter with the higher blue intensity with spinach, Xanthium and soybean.

The dry weight data as given in the tables for the blue plot is in several instances higher than the control and blue 4X plots. This is especially true of the aster and Scabiosa data. This condition was possibly caused by the slightly higher air temperature in the end of the greenhouse where the blue plot was located. The air temperature for this plot occasionally ran several

FIG. 2. *Callistephus chinensis*, variety Heart of France. Photographed after 71 days of irradiation.

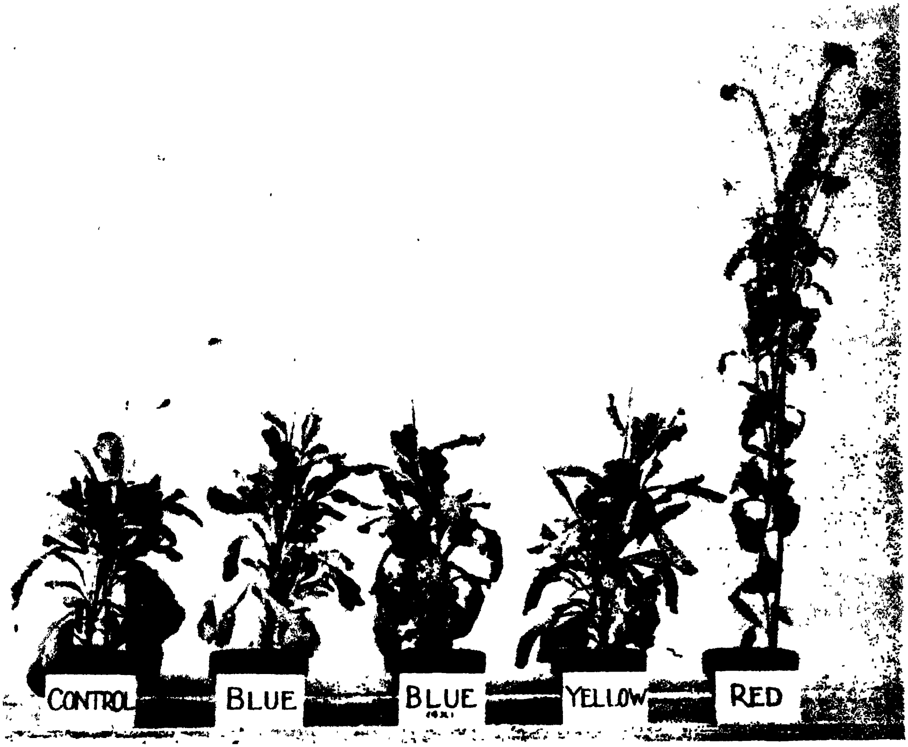


FIG. 3. *Scabiosa atropurpurea*, variety Azure Fairy. Photographed after 81 days of irradiation.

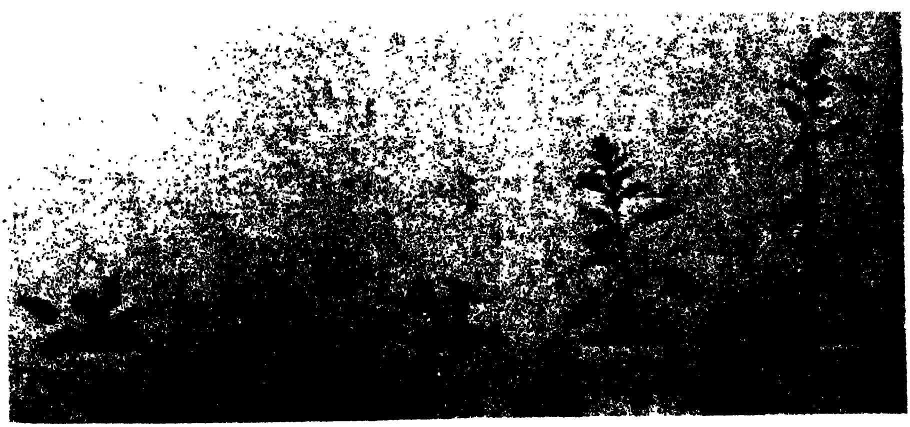


FIG. 4. *Spinacia oleracea*, variety Nobel. Photographed after 37 days of irradiation.

FIG 5 *Soja max*, variety Mukden Photographed 73 days after seeding.

degrees higher than that for the others owing to a door opening into an adjacent warmer house

An increase in height occurred under the red radiation with all the plants used in the investigation The greatest increase occurred with the long day plants The aster and spinach in the short day treatment remained in rosette form, while the red and yellow-green radiation brought about marked increases in height; in the case of spinach this amounted to as much as 80 to 90 times. The relative increase in height in the short day plants was not so great, being of the order of from 0 1 to 3 times.

FIG. 6. *Xanthium pennsylvanicum.* Photographed 64 days after seeding.

The top-root ratio was increased under the red in all cases where weight data were taken, except with Xanthium where the ratio was highest under the short day treatment. With Xanthium, the decreased ratio appeared under the yellow-green and high intensity blue, as well as under the red. Because of this difference in relation to the other plants, a second group of these plants was grown under a 9-hour day and under a 9-hour day supplemented with 15 hours of red radiation. The data for these are presented in table IX. The red radiation again caused a lower top-root ratio than did the short day condition. In aster, spinach, and soybean the top-root ratio was increased by the yellow-green and blue radiation treatments, but not to the degree occurring under the red.

Aster showed a decreased number of leaves under the red, yellow-green and high intensity blue and a marked increase in leaf size, progressively through the blue, high intensity blue, yellow-green, and red. Leaf area also was increased to the greatest extent under the red, although all the supplemental radiations had some effect.

In soybean, there was no significant variation in number of leaves per plant among the various plots at the time of harvest, but there was an increase in leaf area under the supplemental light treatments in the same progressive order as for aster but the percentage increase was not so great as with aster.

In the case of Tithonia, it is interesting to note that the blue has an inhibitory effect on the number of flower buds, the high intensity blue having a greater inhibitory effect than the low.

Discussion

These results are quite consistent in indicating that it is the longer wavelengths of the visible spectrum which are chiefly responsible for the photoperiodic response of both long and short day plants to long day treatments. The red is apparently somewhat more effective than the yellow-green. The blue is only slightly influential in altering the response of the plant from that obtained in the short day condition. In general, height, weight, top-root ratio, and the percentage of dry matter all point to the conclusion that the longer wavelengths also induce the vegetative responses associated with the long day treatment.

Since the blue has little influence in both inhibition of flowering in short day plants and flower induction in long day plants, it appears that the blue radiation is not strongly absorbed by the photo-activated molecule involved in the photoperiodic system since only that radiation which is absorbed can be effective. Therefore, it must be assumed either that the absorbing molecule has strong absorption bands in the red, weaker bands in the yellow-green, and very weak bands in the blue, or that the blue bands are relatively

strong, but that a blue absorbing system such as the carotenoids is exhibiting a filtering effect. The latter hypothesis appears rather unlikely since, while the carotenoids are a part of the pigment system of the chloroplasts, they do not produce a sufficiently strong filtering effect to greatly reduce the absorption of blue radiation by the chlorophyll. Photosynthetic activity and chlorophyll absorption show maxima of the same order of magnitude in the blue and the red. It hardly seems likely that the carotenoids would exert any stronger filtering effect in the case of photoperiodic processes.

Assuming negligible filtering effect, the general region of maximum absorption within the visible spectrum of the photo-activated molecular species involved in the mechanism is in the red, with a somewhat decreased absorption in the yellow-green, and a greatly reduced absorption in the blue. The photo-activated molecule involved in photoperiodism is probably the same for both long and short day plants since the same general regions of radiation are effective in producing their respective responses.

Since the leaves of the plant have been identified as the principal loci for radiation absorption leading to photoperiodic flower induction and inhibition (**2, 6, 7**), it would appear that there is a pigment present in the leaves which has a region of maximum absorption in the red and a minimum in the blue. That this pigment must be present in all of these plants, regardless of their flowering response to daylength, is postulated from the results which show that the effective and therefore absorbed radiation for the plants investigated, both of the long and short day type, is in the longer wavelengths.

The results do not point beyond the initial absorption of radiant energy. It should be pointed out that the molecule absorbing the radiation is not necessarily involved in the series of steps leading to the eventual photoperiodic response further than the transfer of energy to activate other molecules. What type of reaction within the plants which leads to the induction of flower buds in the one case and the production of foliar buds in the other is not indicated.

The hypothesis presented herein as to the similar nature of the absorption reaction involved in the photoperiodic responses of long and short day plants is based upon results obtained with only three relatively wide bands of radiation of equal energy in the visible spectrum. Studies must be made at many more points in the visible spectrum with very narrow bands or monochromatic radiation before conclusive evidence can be presented as to the absorption spectrum of the photo-activated substance.

Summary

Three long day plants, *Callistephus chinensis*, variety Heart of France, *Scabiosa atropurpurea*, variety Azure Fairy, *Spinacia oleracea*, variety Nobel, and four short day plants, *Soja max*, variety Mukden, *Xanthium*

pennsylvanicum, Salvia splendens, variety Fairly Tall, and *Tithonia speciosa* were grown in subirrigation nutrient solution culture. They were given natural short winter days and days lengthened to 24 hours by blue radiation (4047 and 4358Å), yellow-green (5770 and 5791Å), and red (6400-9000Å) of approximately 100 ergs/cm.^{2}/second, and blue of 400 ergs/cm.^{2}/second.

All the long day plants set macroscopic flower buds under red and yellow-green radiation; spinach and Scabiosa failed to set buds under blue or the short day condition. China aster, which was grown into the longer spring days, flowered under a 12-hour daylength, the blue radiation having only a slight effect in reducing flowering time.

All the short day plants flowered under the short day condition and blue radiation, but failed to set buds under either red or yellow-green.

The red radiation brought about an increase in dry matter in all the plants studied. In some cases, the yellow-green and blue radiations also brought about an increase in total dry weight. Except with Scabiosa, the red and yellow-green caused a decrease in the percentage of dry matter, however. The higher blue radiation also caused a decreased percentage of dry matter with spinach, Xanthium, and soybean.

Height was increased under the red and yellow-green with all the plants, the increase being of much greater magnitude with the long day plants than with the short day ones.

Top-root ratio increased under the longer wavebands in all cases with the exception of Xanthium where a decrease in ratio was noted. With aster, spinach, and soybean, the ratio was increased also by the blue but not to the extent occurring under the red.

Leaf area for aster and soybean was increased progressively from the short day through the blue, high intensity blue, yellow-green, and red.

The results secured in this investigation indicate that it is the longer wavelengths of the visible spectrum which are primarily effective in producing the flowering and vegetative effects secured under long day treatments with both the long and short day plants used. The initial photo-chemical step appears to be similar for all the plants tested.

The authors wish to express their sincere appreciation to Mr. ROBERT K. SHOWALTER and Mr. THOMAS EASTWOOD for their assistance with the experimental work.

INDIANA AGRICULTURAL EXPERIMENT STATION
WEST LAFAYETTE, INDIANA

LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W. Photoperiodic perception in Biloxi soybeans. Bot. Gaz. **100**: 374–387. 1938.

2. CAJLACHJAN, M. H. On the mechanism of photoperiodic reaction. Compt. Rend. Acad. Sci. U.R.S.S. **1**: 89–93. 1936.
3. FUNKE, G. L. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **3**: 225–261. 1936.
4. ————. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **4**: 345–359. 1937.
5. ————. Proeven over photoperiodiciteit by verschillend gekleurd licht. Biol. Jaarboek Nat. Genoot. Dodonaea **5**: 404–424. 1938.
6. HAMNER, K. C., and BONNER, JAMES. Photoperiodism in relation to hormones as factors in floral initiation and development. Bot. Gaz. **100**: 388–431. 1938.
7. KNOTT, J. E. Effect of a localized photoperiod on spinach. Proc. Amer. Soc. Hort. Sci. **31**: 152–154. 1934.
8. RASUMOV, V. The significance of the quality of light in photoperiodical response. Bul. Appl. Bot., Gen. and Plant Breeding, III. Phys., Biochem. and Anat. Plants **3**: 217–251. 1933.
9. SCHAPPELLE, N. A. Effect of narrow ranges of wavelengths of radiant energy, and other factors, on the reproductive growth of long day and short day plants. New York (Cornell) Agr. Exp. Sta. Mem. 185. 1936.
10. WITHROW, R. B. A photoelectric device for the rapid measurement of leaf area. Jour. Agr. Res. **50**: 637–643. 1935.
11. ————, and BENEDICT, H. M. Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daily period. Plant Physiol. **11**: 225–249. 1936.
12. ————, and BIEBEL, J. P. Photoperiodic response of certain long and short day plants to filtered radiation applied as a supplement to daylight. Plant Physiol. **11**: 807–819. 1936.
13. ————, and ————. Nutrient solution methods of greenhouse crop production. Indiana Agr. Exp. Sta. Cir. **232**: 1–20. 1938.

EFFECTS OF ENVIRONMENTAL FACTORS ON OXIDIZING ENZYMES OF ROSE MALLOW SEEDS

KATHRYN STALEY

(WITH EIGHT FIGURES)

Introduction

The power of plant tissues to break up hydrogen peroxide into water and molecular oxygen was known long before catalase was recognized as an enzyme, discovered, and named by LOEW (18) in 1901. This discovery opened a new field for investigation and many studies were undertaken. The wide distribution of catalase in plant and animal tissue led LOEW to conclude that it might serve as a protective measure against the injurious protoplasmic effects of hydrogen peroxide and possibly other related compounds formed in respiration. DIXON (8) has shown that when purine bases are oxidized by molecular oxygen in the presence of xanthine-oxidase as a catalyst, the oxidase undergoes destruction during the course of the reaction owing to the hydrogen peroxide that is formed. He found that this destruction could be prevented by the addition of catalase. Pneumococci in contact with a free supply of oxygen produced hydrogen peroxide in sufficient amount to inhibit their growth, but if catalase were added the bacteria grew vigorously.

A close correlation between catalase activity and respiration was found in potatoes (2), in rice grains germinated under water (22), and in sweet corn (3). CROCKER and HARRINGTON (6) found this same correlation in the seeds of Johnson grass but not in Amaranthus. RHINE (26) using a number of different seeds concluded that catalase activity is a measure of the metabolic activity only when there is a rapid change in respiration. The absence of catalase activity in certain blue green algae (14) and in anaerobic bacteria (20) indicates that catalase is not essential to the respiration of all cells. MCLEOD and GORDON (20) consider it possible that catalase destroys some injurious substance such as H_2O_2 which is produced by aerobic organisms and by anaerobic organisms in the presence of oxygen; thus anaerobic organisms lacking catalase would be injured when exposed to oxygen.

A variation in catalase activity was observed by POPE (24, 25) in the different parts of the same barley plant. The relation of the catalase activity to physiological breakdown in Jonathan apples convinced NELLER (23) that catalase activity could be used as a true index of the rate of metabolic activity. HEINICKE (15, 16) concluded that catalase activity is a highly sensitive index to changes in the internal condition of the apple tree, and that this activity varies with the season, with the part of the tree tested, and with

various environmental factors. He believed that the catalase activity would eventually prove as good an indicator of metabolism as do the temperature and pulse rate in the human body.

The detailed nature of catalase has been carefully investigated. LOEW (18) recognized an insoluble and a soluble form. He thought the insoluble form, or alpha catalase, was a compound of the soluble form with a nucleoprotein. The soluble form, beta catalase, was considered an albumose which could be liberated by the action of a very dilute alkaline medium upon the insoluble form. APPLEMAN (1) was able to separate the two forms by the use of ordinary filter paper, but could not do so with a Chamberland-Pasteur filter. Approximately fifty per cent. of the total enzyme content passed through the filter paper, indicating that the two forms were present in about equal amounts.

For the chemical analysis of catalase, liver has served as the most convenient source of the catalase preparation. EULER and JOSEPHSON (11) state that no relationship exists between the activity of the preparation and the percentage of iron present. They analyzed two preparations which had a catalytic activity of 43,000 and 40,000 respectively, and found that the former contained 0.63 per cent. iron while the latter contained 0.15 per cent. ZEILE and HELLSTRÖM (32) found that a strong preparation of catalase obtained from horse liver had a distinct absorption spectrum corresponding to a hematin compound. Regardless of degree of purity they thought there was a strict proportionality between the catalytic activity and the concentration of the porphyrin-bound iron. Comparing the value calculated for hemin with that for catalase they found that in the catalase union the iron was decidedly more active than the usual porphyrin-bound iron. They concluded that the porphyrin-iron complex in catalase must be bound in a very specific way to the protein or some other colloidal carrier, which binding confers to it this unusual increase of activity. They also found that such factors as high temperature, alkali, acids, or the addition of small amounts of KCN or H_2S, which abolished or inhibited the catalase activity, also modified the absorption spectrum of the hematin. STERN (27) studying the activity of ferrous and ferric ions, hemin, and catalase, respectively, in decomposing H_2O_2, found that the binding of iron into porphyrin resulted in a greater catalytic activity than for iron alone; and a still greater activity was obtained when hemin was coupled with catalase protein. The absorption spectrum and pH observations led STERN (27, 28) to conclude that the parahematin compounds were closely related to natural catalase. It has been estimated that the diameter of a catalase particle is 5.46 mμ and that the molecular weight of catalase is approximately 68,900. This indicates that the molecules are similar in size to those of hemoglobin. Horse liver catalase has been split by dialysis into two inactive components, one of low molecular

weight (possibly hemin) and the other possibly protein, which, upon being mixed become active. TAUBER and KLEINER (30) concluded that catalase solutions could be digested by trypsin.

Crystalline catalase prepared from beef liver (29) coagulated upon heating and showed many other properties characteristic of proteins. The investigators report that the pyridine hemochromogen test was readily obtained with this preparation. It appears, then, that catalase is similar to peroxidase (13) and oxidase (13, 31, 44) in having an iron-porphyrin complex as the active group.

The catalase studies reported here were undertaken to determine the effect of environmental factors such as temperature, exposure to gases, and the submergence of seeds in water upon the activity of this iron porphyrin complex of imbibed seeds. Other oxidizing enzymes also were studied to obtain a more generalized idea of the effect of such conditions on the enzyme complexes associated with oxidation in the plant.

In biological oxidations, peroxidases are thought to transfer the oxygen, linked as a peroxide, to oxidizable substances. They cannot decompose H_2O_2 in the absence of an oxidizable substance. The oxidases render such matter more susceptible to reduction.

Materials and methods

Seeds of the Malvaceae are noted for their hardness and longevity. Two varieties of rose mallow, or Hibiscus, were selected: the variety Golden Bowl, and variety Dark Red. The seeds of the former are larger than those of the latter and are approximately twice as heavy; both are very erratic in germination because of their impervious seed coats. Seeds of the 1936 crop of Dark Red and of the 1934 and 1936 crop of Golden Bowl were obtained from Henry Dreer Seed Co., Philadelphia, Pa. Only a small quantity of the 1936 crop of Golden Bowl was available.

In order to secure greater uniformity in germination the seeds were subjected to the action of H_2SO_4 (sp. gr. 1.84) for 53 minutes, washed with sterile water, and the acid neutralized with lime water. They were placed in sterile water until they showed evidence of swelling, disinfected with 0.25 per cent. solution of uspulin for one hour, and thoroughly washed with sterile water. The usuplin treatment was inadequate for the seeds which were kept under water for 30 days, consequently, a more effective method of disinfection was used. This treatment consisted of soaking the seeds 4 hours in a 1:30 dilution of Zonite and rinsing in sterile water before placing them under the conditions of the experiment.

All glassware, cellucotton, filter paper, and water used for the experiments were sterilized in the autoclave at 15 lb. pressure for 30 min. on each of two successive days. A modified APPLEMAN (1) apparatus was used for

catalase determinations; the liberation of oxygen from neutralized Merck's hydrogen peroxide served as a measure of the catalase activity. Each bottle was standardized by permanganate titration before using, and the equivalent of 10 ml. was used for each determination. The tissue was ground for 5 min. in a mortar with clean sand, a small excess of $CaCO_3$, and a measured amount of distilled water. The material was then washed into the shaking bottle with the remainder of the water previously measured. The equivalent of 10 ml. of water was used for each determination. The bottle was fastened in the shaker in such a manner that the former was suspended in a water bath maintained at a temperature of 20° C. When the contents of the bottle were of the same temperature as the water bath, the two-holed stopper fitted with dropping funnel and delivery tube was adjusted to position, and 10 ml. of neutralized H_2O_2 was added 15 seconds before the shaking began. The levelling bulb was used to maintain pressure equilibrium. Readings were taken at 1, 2, 3, 5, and 10 minutes. The first four readings served as a check on the final reading and showed the rate at which the molecular oxygen was evolved. The results were corrected for standard conditions of temperature and pressure.

Simplified Bunzell tubes and a mechanical shaker were used for oxidase determinations. The tissues were ground for 5 min. in a mortar with a very small quantity of distilled water. The ground tissue was then washed into a small graduated calibrated cylinder. The volume was brought to 4 ml. and the contents thoroughly mixed. One ml. of this mixture was pipetted into the short arm, and 5 ml. of a 1 per cent. solution of pyrogallol into the larger arm, of the Bunzell tube; care was taken not to mix the two until the shaker was started. The top was adjusted to leave the contents open to the outside air, while the tube was fastened into the mechanical shaker and allowed to stand until temperature equilibrium had been established: The top was then turned to prevent contact with the outside air and the shaker, adjusted to make one hundred complete excursions per minute, set in motion. Readings were made at 15-minute intervals for the first hour and at thirty-minute intervals for the second hour. At the end of the first hour the manometers were opened and reset. The Bunzell tubes were calibrated, and the results corrected for 25 ml. volume and standard conditions of temperature and pressure.

Results

Temperature effects on catalase

Imbibed seeds of the two varieties tested were placed on moist cellucotton and filter paper in sterile Petri dishes. The seeds were subjected to temperatures of 8°, 20°, 37°, 42°, and 47° C. for a period of seven days. Catalase determinations in duplicate were made daily. Six seeds of variety Golden Bowl

and twelve seeds of Dark Red, respectively, were used in a determination. All results were corrected to standard conditions.

Seeds of the 1934 crop of Golden Bowl subjected to temperatures of 8°, 20°, and 27° C. show a decrease in catalase activity, followed by an increase until the food is exhausted, at which time the catalase decreases (table IA).

TABLE I

TEMPERATURE EFFECTS ON CATALASE ACTIVITY OF HIBISCUS SEEDS. MILLILITERS OF OXYGEN LIBERATED IN TEN MINUTES

	DAYS	TEMPERATURE					
		8° C.	20° C.	27° C.	37° C.	42° C.	47° C.
		ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
A	0	39.6	39.6	39.6	39.6	39.6	39.6
	1	41.2	32.1	34.4	40.2	30.2	0.9
	2	40.3	40.5	34.8	40.2	9.7	0.0
Golden Bowl	3	29.7	71.0	66.9	36.3	14.8	0.0
Six seeds of 1934 crop	4	33.6	52.8	36.0	31.5	4.5	0.0
	5	42.6	76.6	55.4	14.2	4.3	0.0
	6	44.6	82.8	79.0	6.0	1.9	0.0
	7	45.9	55.6	97.1	4.8	1.0	0.0
B	0	36.6	36.6	36.6	36.6	36.6	36.6
	1	40.3	40.9	35.9	29.3	15.6	0.5
	2	32.6	33.9	34.6	28.2	16.8	0.4
Golden Bowl	3	33.0	38.4	45.4	15.5	7.1	0.4
Six seeds of 1936 crop	4	29.3	42.7	45.6	14.1	5.7	0.3
	5	32.2	48.5	65.9	13.4	4.7	0.2
	6	28.3	59.5	109.6	13.5	2.2	0.1
	7	30.9	69.8	93.5	5.8	1.4	0.1
C	0	38.8	38.8	38.8	38.8	38.8	38.8
	1	26.6	19.4	20.6	22.4	27.0	0.0
	2	28.0	46.2	57.4	35.5	19.5	0.0
Dark Red	3	39.7	46.8	35.1	31.8	4.8	0.0
Twelve seeds of 1936 crop	4	23.9	71.7	97.5	38.4	12.9	0.0
	5	22.6	91.5	105.3	38.5	8.6	0.0
	6	19.2	97.6	104.7	33.1	6.4	0.0
	7	24.5	109.4	86.5	29.5	4.9	0.0

The drop in catalase content is slower in the seeds kept at 8° C. since it takes place on the fourth day; at 20° and 27° C. the depression occurs on the first day. At 37° C. there is noted a very slight increase on the first and second day (the depression may have come earlier than the 24-hour limit) followed by a gradual decrease in catalase content. The seeds at this temperature gave no visible signs of germination. At 42° C. there is a slight decrease on the first day followed by a rapid drop in catalase activity. At 47° C. the decrease the first day is very rapid and soon reaches zero.

The catalase activity of the 1936 crop of variety Golden Bowl agrees rather closely with that of the 1934 crop (table IB). At 47° C. the catalase activity shows a slightly greater resistance to this temperature than that

of the 1934 crop. The results obtained indicate that the difference in age in these particular seeds is of no significance in catalase activities.

The seeds of variety Dark Red and Golden Bowl respond in a similar fashion in both germination and catalase activity (table IC, fig. 1, 2).

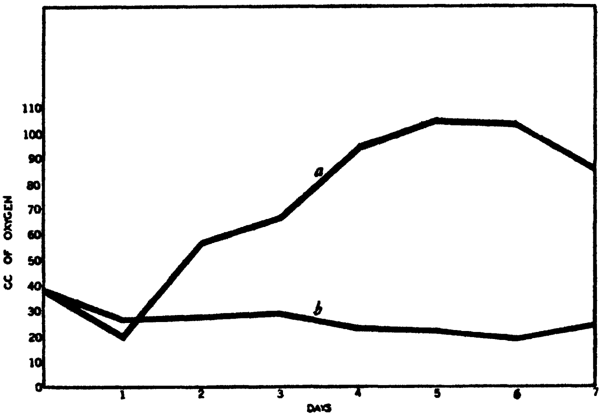


FIG. 1. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 27° C., and (b) 8° C.

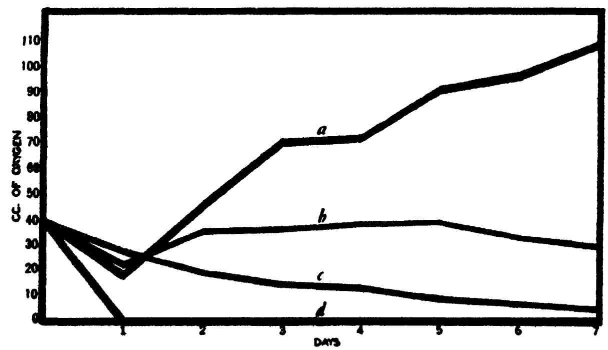


FIG. 2. The effect of temperature on the catalase activity of imbibed seeds of Hibiscus variety Dark Red. (a) 20° C., (b) 37° C., (c) 42° C., and (d) 47° C.

At 8°, 20°, 27°, and 37° C. there is a characteristic decline in catalase activity in the early stages of germination, followed by an increase. This decrease occurs later with seeds kept at 8° C. than with those at the higher temperatures. Good germination occurred at 20° and 27° C., while only a few were able to germinate at 37° C. Apparently this accounts for the greater activity of the catalase at 37° C. as compared with that of the Golden Bowl seeds at the same temperature. At 42° C. the seeds gave no indication of germination and there was a gradual decrease in the catalase activity. At 47° C. the catalase was completely inactivated during the first day.

The results indicate that the catalase activity follows a definite course. In the early stages of germination the activity is depressed, but increases as germination progresses, until the food supply is apparently exhausted.

THE EFFECT OF OXYGEN AND NITROGEN ON CATALASE

In order to determine the effect of oxygen and nitrogen on the catalase activity of imbibed seeds, swollen seeds of both species were placed on moist cellucotton and filter paper in a series of 8-oz. bottles. Each bottle contained a number of seeds sufficient for seven daily determinations. The bottles were connected and one series subjected to a steady stream of oxygen; the second series was subjected to a steady stream of nitrogen. The bottles were kept in the basement at a room temperature of 25°–27° C. Six seeds of Golden Bowl and twelve seeds of Dark Red, (except where indicated) were used in each determination.

TABLE II

CATALASE ACTIVITY OF HIBISCUS SEEDS TREATED WITH OXYGEN. MILLILITERS OF OXYGEN LIBERATED IN MINUTES ON SEVEN CONSECUTIVE DAYS

Days	Minutes									
	Six seeds of Golden Bowl					Twelve seeds of Dark Red				
	1	2	3	5	10	1	2	3	5	10
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	6.7	14.6	20.7	23.0	39.6	9.2	14.8	19.4	26.8	38.8
1	5.7	11.8	15.7	20.6	30.4	7.3	11.8	17.1	23.8	34.0
2	10.9	16.9	21.4	28.1	37.6	13.1	22.0	33.3	45.0	69.1
3	12.1	20.9	28.3	39.1	57.1	21.4	35.4	46.9	64.1	89.7
4	11.7	20.6	27.6	38.9	57.8	35.3	60.4	79.0	108.4	152.9*
5	15.1	31.7	36.0	52.6	75.1	34.9	58.1	78.6	109.5	160.8*
6	17.6	28.3	36.9	51.9	76.5	30.1	49.7	64.4	88.7	127.4*
7	6.1	9.9	14.1	20.9	33.7	30.0	49.0	62.9	86.6	118.3*

* Owing to the high catalase activity, 6 seeds were used for each determination and four determinations made so that the number given above is the amount of O_2 evolved by 12 seeds.

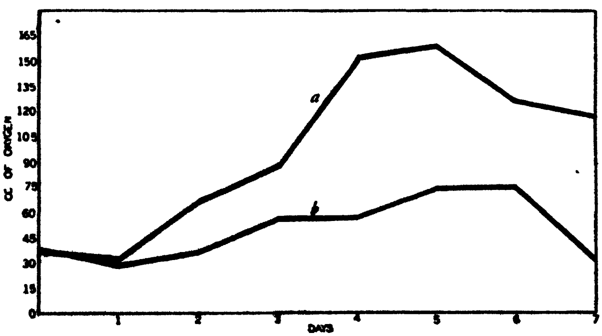


FIG. 3. The effect of oxygen on the catalase activity of imbibed seeds of (a) variety Dark Red and (b) variety Golden Bowl. Based on 10-minute data.

The results given in table II and figure 3 are for 1, 2, 3, 5, and 10 minutes. This shows the rate at which the oxygen is evolved as well as the final reading. The results given indicate that the seeds of the two species treated with oxygen show the same general course of catalase activity which is similar to that obtained in the seeds subjected to temperatures favorable for germination. The seeds of both species show a decrease in catalase on the first day, with an increase reaching the peak for variety Dark Red on the 5th day and for Golden Bowl on the 6th day, followed by a decrease. The conditions were favorable for good germination.

The results of the treatment with nitrogen are essentially the same as those obtained with oxygen (table III, fig. 4). The amount of increase in

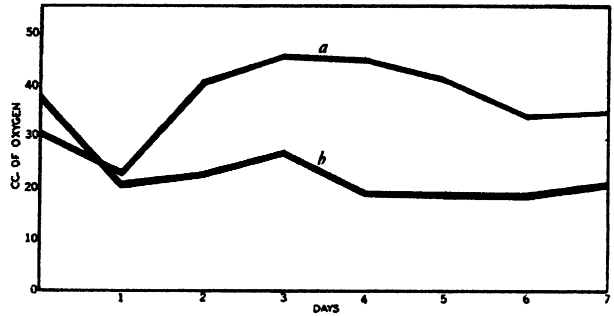


FIG. 4. The effect of nitrogen on the catalase activity of imbibed seeds (a) Golden Bowl and (b) Dark Red. Based on 10-minute data.

catalase activity is less, however, and it was not completely inactivated during the seven day period. Only the initial stages of germination were noted since the absence of oxygen precluded the possibility of the usual reaction.

TABLE III

CATALASE ACTIVITY OF HIBISCUS SEEDS TREATED WITH NITROGEN. MILLILITERS OF OXYGEN LIBERATED IN MINUTES ON SEVEN CONSECUTIVE DAYS

DAYS	MINUTES									
	SIX SEEDS OF GOLDEN BOWL					TWELVE SEEDS OF DARK RED				
	1	2	3	5	10	1	2	3	5	10
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	6.7	14.7	20.7	23.0	31.0	9.2	14.8	19.4	26.8	38.8
1	4.9	10.3	13.6	19.0	23.8	4.9	8.4	11.6	15.6	21.3
2	11.0	18.7	25.0	32.5	40.8	5.1	8.7	12.2	16.7	23.1
3	12.1	20.6	27.2	35.6	45.5	5.2	9.5	13.1	18.8	27.3
4	6.8	14.7	19.6	29.6	45.3	4.5	7.7	10.3	14.1	19.4
5	7.6	14.8	21.3	33.7	40.9	4.0	6.6	9.6	13.6	19.2
6	7.2	13.2	18.4	25.5	34.4	3.7	6.7	9.3	13.6	19.4
7	6.9	11.7	16.4	23.1	35.0	3.6	6.7	9.4	15.3	20.8

Catalase activity of submerged seeds

Imbibed seeds were placed in a series of plugged tubes containing sterile distilled water to a depth of 5, 100, and 155 mm. The seeds were kept at room temperature for a period of thirty days and catalase determinations were made daily. Six seeds of Golden Bowl and 12 seeds of Dark Red were used in the determinations. Under all conditions there was a decrease in catalase activity in the early part of the time period then a slight increase followed by a marked decrease (table IV). Figure 5 shows the course of the catalase activity of the 1934 crop of Golden Bowl at the various depths. The embryos were able to undergo the early stages of germination and a few pushed the radicle through the seed coat. The rate and amount of decrease

TABLE IV

Catalase activity of Hibiscus seeds submerged in water. Milliliters of oxygen liberated in ten minutes on thirty consecutive days

Days	Golden bowl, 6 seeds						Dark Red, 12 seeds		
	1934 crop			1936 crop			1936 crop		
	Water depth			Water depth			Water depth		
	5 mm.	100 mm.	155 mm.	5 mm.	100 mm.	155 mm.	5 mm.	100 mm.	155 mm.
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0	45.0	45.0	45.0	47.0	47.0	47.0	39.0	39.0	39.0
1	35.6	44.4	28.3	30.8	40.4	37.7	22.6	13.8	21.5
2	45.7	37.3	39.7	36.1	39.6	41.7	25.0	23.0	23.7
3	44.5	46.6	50.0	43.4	40.4	29.6	19.7	20.7	22.2
4	42.8	50.5	41.7	41.7	40.5	42.4	30.4	20.9	21.9
5	42.1	43.2	56.5	34.5	39.2	37.6	26.9	33.6	24.9
6	53.3	41.9	42.1	27.6	43.7	37.5	21.5	25.4	19.3
7	43.6	37.4	43.1	43.6	41.7	39.7	22.8	16.3	18.2
8	33.6	33.9	36.3	43.6	34.5	32.9	23.2	27.5	21.3
9	27.5	19.6	44.0	48.5	33.2	38.6	22.7	20.2	21.1
10	31.5	32.8	43.9	44.7	36.3	40.1	20.9	20.2	17.4
11	34.4	19.9	22.9	44.1	34.5	41.3	21.9	15.8	22.8
12	39.8	18.2	16.4	48.3	38.3	34.9	21.9	14.6	18.1
13	33.7	18.0	19.8	44.9	33.8	34.1	20.8	14.6	19.2
14	33.8	18.5	23.9	38.2	33.7	37.1	21.8	12.1	15.9
15	41.6	13.4	19.6	36.2	30.0	30.5	20.3	12.3	15.1
16	37.7	13.5	8.8	39.9	16.9	26.7	21.1	23.9	16.2
17	25.2	15.5	9.8	40.1	13.8	21.7	16.5	19.5	15.4
18	21.4	18.4	9.0	42.2	12.2	18.1	27.2	16.9	17.3
19	18.9	15.5	10.1	27.9	18.6	11.9	25.1	11.8	21.4
20	22.1	9.4	13.6	27.7	17.4	3.2	24.4	12.2	18.2
21	20.4	13.4	9.9	30.0	19.2	6.9	20.7	22.4	13.8
22	18.9	14.1	5.5	32.8	23.8	10.2	18.5	9.2	12.9
23	27.2	14.4	1.4	32.1	29.7	7.1	18.4	15.6	9.0
24	16.8	14.3	2.9	31.6	19.9	3.3	17.3	14.6	12.2
25	16.3	12.2	2.3	25.0	17.4	6.6	20.7	15.3	15.1
26	17.5	10.4	3.6	15.7	3.6	5.3	21.2	13.5	14.6
27	17.8	11.3	3.7	27.9	5.8	3.4	19.6	13.6	9.4
28	8.3	7.3	2.6	27.1	4.1	2.0	26.8	10.3	13.7
29	13.6	4.8	0.3	28.9	2.9	1.7	19.4	7.6	17.5
30	13.4	0.7	0.2	21.1	1.0	0.2	21.7	14.8	16.6

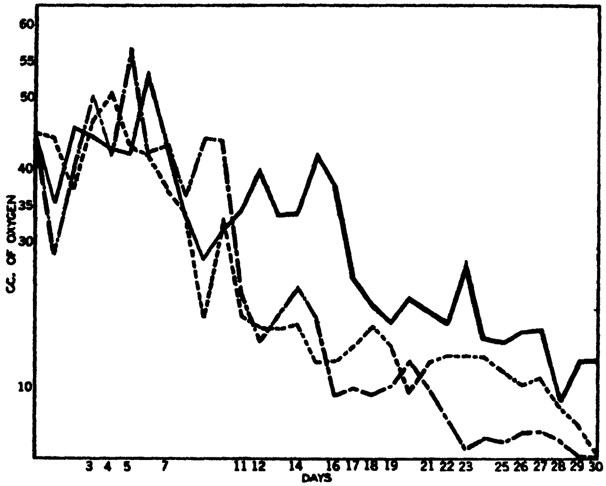


FIG. 5. The effect of a limited supply of oxygen on the catalase activity of Golden Bowl, 1934 crop. Solid line represents imbibed seeds immersed under 5 mm. of water, line of short dashes seeds immersed under 100 mm., and line of long and short dashes seeds under 155 mm.

was evidently correlated with the oxygen supply. The seeds submerged at a depth of 100 mm. and 155 mm. showed a much greater decrease of catalase activity over the later period of the experiment than did those at a depth of 5 mm. In all cases the decline in the catalase activity was well under way in the seeds subjected to 100 mm. and 155 mm. by the fifteenth day. The Golden Bowl seeds of the 1934 crop, at all three depths, showed at some stage in the course of the experiment a greater catalase activity than was present at the beginning. This was shown by the seeds of the 1936 crop of the same species at the 5-mm. depth and not at all by the seeds of variety Dark Red.

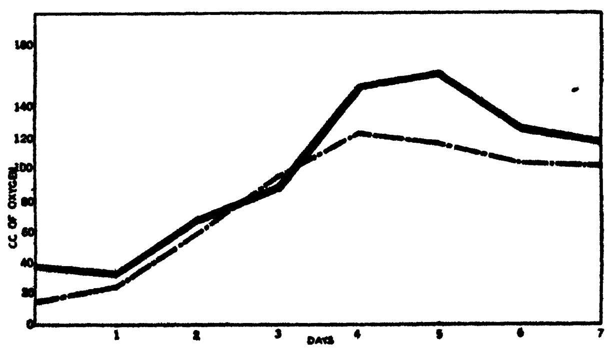


FIG. 6. The catalase activity of seeds of variety Dark Red treated with oxygen: solid line represents imbibed seeds, and the broken line imbibed and held under 155 mm. of water for 20 days before testing.

At the end of the thirty-day period the seeds of this variety showed a greater catalase activity than did those of Golden Bowl.

Seeds of variety Dark Red after being submerged thirty days under a 155-mm. layer of water were removed and placed under ideal germinating conditions with a steady stream of oxygen passing over them; the seeds thus treated gave a 50 per cent. germination. Catalase determinations of the germinating seeds were made on seven consecutive days. Figure 6 shows a comparison of the catalase activity of seeds submerged for 30 days, and of imbibed seeds when placed under oxygen. The submerged seeds show an increase in catalase activity and follow the same general pattern as do the imbibed seeds.

Temperature effects on oxidase

Imbibed seeds were placed on moist filter paper and cellucotton in sterile Petri dishes and subjected to 8° and 47° C. for a period of 7 days. Oxidase determinations were made daily using 12 seeds of the Golden Bowl and 24 seeds of Dark Red. The results show (table V) that oxidase, like catalase, is destroyed or inactivated at 47° C. The amount of oxidase fluctuates at 8° C., showing no great increase, and reaches its lowest level on the fourth day in the seeds of Golden Bowl and on the seventh day in those of Dark Red.

TABLE V

Temperature effects on oxidase activity of Hibiscus seeds. Manometer readings expressed in centimeters of mercury for two hours on seven consecutive days

Days	Golden Bowl, 12 seeds		Dark Red, 24 seeds	
	8° C.	47° C.	8° C.	47° C.
	cm.	*cm.*	*cm.*	*cm.*
0	−0.51	−0.51	−0.49	−0.49
1	−0.25	−0.00	−0.27	−0.00
2	−0.41	−0.00	−0.21	−0.00
3	−0.21	−0.00	−0.33	−0.00
4	−0.14	−0.00	−0.29	−0.00
5	−0.20	−0.00	−0.13	−0.00
6	−0.19	−0.00	−0.15	−0.00
7	−0.33	−0.00	−0.07	−0.00

Effect of oxygen and nitrogen on oxidase

The imbibed seeds were placed in bottles in the same manner as in the catalase experiment and subjected to identical conditions. Daily determinations were made using 12 of the larger variety and 24 seeds of the smaller variety as in the temperature tests.

The seeds subjected to the steady stream of oxygen showed rapid germination, but the oxidase content seemed to fluctuate during the early part of the experiment. The oxidase activity reached its maximum degree in

Golden Bowl seeds on the 6th day and in the seeds of Dark Red on the 5th day. The results indicate that the oxidase content does vary with germination of the seeds.

TABLE VI

OXYGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

SEEDS	DAYS							
	0	1	2	3	4	5	6	7
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Golden Bowl (12)	– 0.51	– 0.22	– 0.37	– 0.41	– 0.30	– 0.33	– 0.72	– 0.37
Dark Red (24)	– 0.49	– 0.24	– 0.36	– 0.24	– 0.52	– 0.92	– 0.68	– 0.61

Only a few of the Golden Bowl seeds showed any visible signs of germination when subjected to nitrogen and the oxidase content, or activity, showed great fluctuation. The seeds of Golden Bowl showed a greater oxidase activity on the 5th day than did those treated with oxygen; this was not true for variety Dark Red although the high point occurred on the 5th day as it did in the oxygen-treated seeds.

TABLE VII

NITROGEN EFFECTS ON OXIDASE ACTIVITY OF HIBISCUS SEEDS. MANOMETER READING EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON SEVEN CONSECUTIVE DAYS

SEEDS	DAYS							
	0	1	2	3	4	5	6	7
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Golden Bowl (12)	– 0.51	– 0.23	– 0.67	– 0.15	– 0.75	– 0.94	– 0.41	– 0.41
Dark Red (24)	– 0.49	– 0.16	– 0.44	– 0.24	– 0.16	– 0.60	– 0.52	– 0.56

THE OXIDASE ACTIVITY OF SUBMERGED SEEDS

Imbibed seeds were placed in tubes containing sterile water as in the catalase experiment and under identical conditions. Twelve seeds were used for the daily oxidase determination of Golden Bowl and 24 seeds for the other species. In both varieties the first part of the period was marked by considerable fluctuation of oxidase activity in the seeds at all three depths. After the 15th day the oxidase activity decreased consistently.

TABLE VIII

OXIDASE ACTIVITY OF HIBISCUS SEEDS SUBMERGED IN WATER. MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY FOR TWO HOURS ON THIRTY CONSECUTIVE DAYS

DAYS	GOLDEN BOWL, 12 SEEDS			DARK RED, 24 SEEDS		
	1934 CROP			1936 CROP		
	WATER DEPTH			WATER DEPTH		
	5 MM.	100 MM.	155 MM.	5 MM.	100 MM.	155 MM.
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
0	−0.51	−0.51	−0.51	−0.49	−0.49	−0.49
1	−0.48	−0.67	−0.71	−0.75	−0.46	−0.63
2	−0.41	−0.31	−0.37	−0.83	−0.10	−0.23
3	−0.42	−0.64	−0.41	−0.41	−0.06	−0.39
4	−0.30	−0.25	−0.30	−0.79	−0.51	−0.35
5	−0.25	−0.57	−0.25	−0.41	−0.25	−0.35
6	−0.34	−0.24	−0.22	−0.75	−0.61	−0.27
7	−0.30	−0.40	−0.28	−1.13	−0.25	−0.35
8	−0.64	−0.40	−0.22	−1.00	−0.51	−0.46
9	−0.68	−0.16	−0.18	−0.67	−0.67	−0.27
10	−0.71	−0.28	−0.17	−1.34	−0.58	−0.55
11	−0.47	−0.36	−0.14	−0.54	−0.32	−0.59
12	−0.41	−0.24	−0.14	−0.58	−0.34	−0.59
13	−0.52	−0.12	−0.07	−0.40	−0.35	−0.43
14	−0.23	−0.20	−0.17	−0.52	−0.25	−0.07
15	−0.31	−0.36	−0.14	−0.66	−0.19	−0.23
16	−0.20	−0.24	−0.17	−0.54	−0.22	−0.15
17	−0.15	−0.24	−0.17	−0.33	−0.18	−0.15
18	−0.11	−0.28	−0.18	−0.41	−0.29	−0.23
19	−0.22	−0.24	−0.18	−0.66	−0.26	−0.17
20	−0.19	−0.16	−0.17	−0.54	−0.09	−0.15
21	−0.22	−0.24	−0.17	−0.54	−0.15	−0.15
22	−0.27	−0.29	−0.15	−0.58	−0.00	−0.07
23	−0.42	−0.12	−0.14	−0.37	−0.00	−0.15
24	−0.22	−0.16	−0.11	−0.41	−0.18	−0.27
25	−0.29	−0.16	−0.14	−0.16	−0.10	−0.11
26	−0.11	−0.28	−0.14	−0.16	−0.19	−0.11
27	−0.21	−0.08	−0.14	−0.46	−0.28	−0.19
28	−0.19	−0.16	−0.14	−0.50	−0.11	−0.15
29	−0.20	−0.28	−0.14	−0.62	−0.12	−0.15
30	−0.36	−0.24	−0.12	−0.75	−0.19	−0.15

The seeds at the greater depth showed a greater decrease than did those at the 5 mm. depth. Some of the submerged seeds were able to push the radicle through the seed coat. Where the oxidase is present in small amounts, this lack of uniformity in the degree of germination in the individual seeds may be responsible for some of the fluctuation if the oxidase content is influenced by germination as the results indicate (table VI, fig. 7).

Discussion

Catalase activity in the seeds of Hibiscus varieties Golden Bowl and Dark Red is depressed in the early stage of germination and then increases until

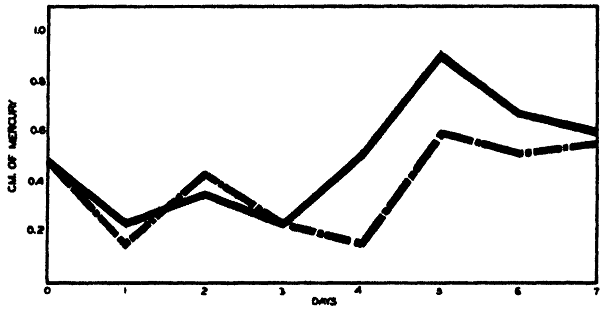


FIG. 7. The oxidase activity of seeds of variety Dark Red: solid line represents the imbibed seeds treated with oxygen, and the broken line seeds treated with nitrogen. The pressures are negative.

the food supply is apparently exhausted. This depression in the catalase activity is not in accordance with the findings of CROCKER and HARRINGTON (6) for grass seeds, but does agree with the work of RHINE (26) for wheat, feterita, clover, mustard, radish, and buckwheat, and of LANTZ (17) for corn. RHINE concluded that catalase increased with the need of the plant to destroy the injurious substances produced in respiration, and thus these by-products stimulated the production of catalase. She explained this decrease in the first stages of germination by assuming that the catalase reserve was used up, in attacking the respiratory products, faster than it

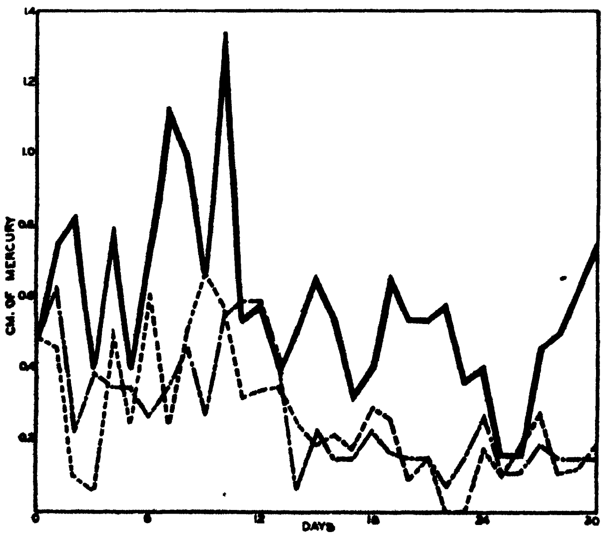


FIG. 8. The effect of a limited supply of oxygen on the oxidase content of imbibed seeds of variety Dark Red; solid line represents seeds immersed under 5 mm. of water, line of short dashes 100 mm., and line of long and short dashes 155 mm. The pressures are negative.

was produced. The increased stimulation of the accumulated respiratory by-products would, in a very short time, result in an increased production of catalase. The time required by the Hibiscus seeds to reach this low point of catalase activity depends on the speed of germination which in turn is dependent upon the external and internal conditions of the seeds.

The effect of the external conditions on the speed and the amount of catalase activity in the Hibiscus seeds is shown in the temperature studies in table 1 and figures 1 and 2. The seeds subjected to 8° C. required a longer period for the decrease in the catalase activity to occur than did those at 20° and 27° C. At the end of the seven-day period at this low temperature there was no evidence of the radicles pushing through the seed coats. The rate of increase of the catalase after the lowest point is reached is very slow in contrast to that in the actively germinating seeds. The lowest point of the catalase activity in the seeds placed at 20° and 27° C. is reached on the second or third day; there is then a sharp increase, reaching the peak during the 5th to 7th day. There is a slight difference in the catalase activity of the two species at 37° C. Variety Dark Red shows a decrease on the second day, with a very slight increase from the 2nd through the 5th, then a gradual decline. A few of these seeds were able to push the radicle through the seed coat at this temperature. The catalase activity of the seeds of Golden Bowl either reached the early decrease before the end of 24 hours or the seeds were unable to germinate at this temperature. At 47° C. in both varieties of seeds there is an abrupt drop in the catalase content beginning with the first day, while at 42° C. the trend is downward but at a slower rate.

The decrease and the lack of increase at the higher temperatures may be attributable to a combined set of factors. At 42° and 47° C. there is no evidence of germination, and since there seems to be some correlation between the germination and rate of catalase activity, this may be responsible for the absence of an increase in catalase activity. On the other hand, catalase shows its similarity to other enzymes in its reaction to several external conditions. There is no one temperature at which inactivation of all enzymes takes place, yet the temperature, time of exposure to heat, and the presence of other substances seem to play an important part in the rate of inactivation (**12, 21**). MORGULIS and BEBER (**21**) state that catalase is slowly inactivated at temperatures below 50° C.; inactivation becomes very rapid as the temperature is raised to 55° C. or above, especially at a pH of less than 6. Considering enzymes in general, FALK (**12**) finds that they show their greatest catalytic activity at temperatures near 40° C. At higher temperatures he thinks it is probable that inactivation of the enzymes takes place with sufficient rapidity to cause an apparent decrease in catalytic action. Investigators using the catalase extract have been able

to subject it to a higher temperature for a short period of time without completely destroying the catalytic activity. This shows the importance of the length of time the enzyme is exposed to a given temperature. An increase in the catalytic activity of a yeast suspension was obtained by EULER and BLIX (10) by heating for one hour at a temperature of 55°–60° C. They attributed this increased activity to the destruction of an inhibitory substance. CROCKER and HARRINGTON (6) heated air dry seeds of *Amaranthus retroflexus* for 4 hours at a temperature of 81° C. with a loss of only 40 per cent. of its original catalase activity, while the vitality of the seed was reduced 75 per cent. They found the reverse to be true for the seeds of Johnson grass. Thus the temperature which will inactivate catalase is determined by the source of the catalase and the conditions under which it is exposed.

The Hibiscus seed subjected to a steady flow of oxygen germinated rapidly at 25°–27° C. and the catalase activity, after decreasing for a short period, increased rapidly as is shown in table II and figure 3. Submerged seeds with a limited supply of oxygen showed similar reaction but there is a decrease in the amount of catalase activity (table IV, fig. 4). This decrease is greater in the seeds submerged at depths of 100 and 155 mm. than at 5 mm. When the oxygen is completely eliminated by passing a steady stream of nitrogen (table III, fig. 5) over the imbibed seeds there is a decrease in the catalase activity of the seeds of Dark Red; in Golden Bowl there is first a decrease, then a slight increase, and finally a second decrease which is typical of germinating seeds. A few of the Golden Bowl embryos were able to push the radicle through the seed coat; none of the Dark Red embryos was able to do so. MARKS and FOX (19) found that catalase extract obtained from muscle, stored under nitrogen, was inactivated at a slower rate than when kept under oxygen or air. It is probable that the effect on the increase of catalase activity is indirect, since germination proceeds rapidly when the seeds are exposed to air or excess oxygen and favorable temperature conditions, while the germination is depressed when the seeds are subjected to a very limited supply of oxygen or none at all. The amount of decrease found over a period of 30 days may be caused by lack of oxygen. When seeds of variety Dark Red were kept under 155 mm. of water for 30 days, then removed and subjected to a steady stream of oxygen and favorable germinating conditions, 50 per cent. of the seeds germinated and the catalase activity of the germinating seeds increased as shown in figure 6. The increase follows the same general course as for imbibed seeds. MORINAGA (22) found that in rice germinated aerobically there was an increase in catalase while in anaerobic germination there was a decrease in the amount of catalase and also in the length of the radicle.

Catalase activity in germinating seeds decreases when the food supply is exhausted according to the findings of LANTZ (17) and DELEANO (7). This is shown to be the case in the Hibiscus seeds. CROCKER and HARRINGTON (6) found that the catalase activity of the embryo of Stoner wheat and Sudan grass was 28 to 29 times that of the endosperm. They explain this by claiming that the endosperm is composed mainly of dead cells with the exception of the aleurone layer. HEINICKE (16) cites the work of AUCHTER and others in which a correlation was found between the nitrogen content of the tissues and its ability to destroy H_2O_2, while an increase in carbohydrate content without further addition of nitrogen depresses catalase. HEINICKE (15, 16) noted that the catalase activity in apple-leaf tissue was influenced by the factors which affected the nutritive or physiological conditions. He found that nitrogenous fertilizers increased the catalase activity of leaves from trees growing in sod. Bark of the roots had a higher activity than did bark from the trunk and the bud tissues were the most active of any on the tree. Strains of corn high in oil content displayed the greatest catalase activity, and the high protein strains ranked second when compared with low fat and low protein strains, according to LANTZ (17). BILETZKY (4) contends that more attention should be given to the factors concerned in the production of the plant and their relation to the catalase content. His findings correspond with others who state that nitrogen fertilizer tends to increase the catalase content. Since catalase apparently increases through the stages in germination when the stored food material is being converted into soluble form, with a subsequent decrease when this material is exhausted, the formation of the catalase may be brought about by the breaking up of the stored food. DOYLE (9) states that there is a temptation to look upon catalase as a labile metabolic by-product rather than as an enzyme with a fundamental physiological function.

The oxidases in general were found to react to the various environmental conditions in much the same way as did the catalase. The amount of oxidase present in the seeds before treatment was negligible, consequently it was necessary to increase the number of seeds used for the determinations. In a seed with such an impervious seed coat oxidation is doubtless reduced to a minimum; thus the imbibed seeds have a greater oxidase content than do the dry seeds. The oxidase increased in the seeds which were able to germinate. CROCKER and HARRINGTON (6) found a greater oxidase activity in coleoptiles and roots than in the whole seeds of Johnson grass, but believed the ratio was no greater than would be expected from the comparison of embryo ends with caryopses.

Oxidase was similar to catalase in its response to low and high temperatures. The oxidases were inactivated at 47° C. by the end of a 24-hour

period. At 8° C. there was no great increase in the amount of oxidase over a period of 7 days.

The seeds subjected to an abundant supply of oxygen showed considerable fluctuation in the oxidase activity during the first few days but reached a very high peak on the 5th day for variety Dark Red and on the 6th day for Golden Bowl. The irregularities found during the first part of the period would undoubtedly be smoothed out with a greater number of determinations. In general, the oxidase increased during the period of germination. When the oxygen supply is limited, or lacking completely, there is a remarkable amount of fluctuation in the oxidase content. The results (table VIII) indicate a greater difference in the oxidase content in seeds of variety Dark Red than in those of Golden Bowl when the supply of oxygen is limited. At the shallower submerged depth, seeds of variety Dark Red showed a much higher oxidase content than when kept at greater depths, and at all depths oxidase decreased considerably after the 11th or 12th day. When the supply of oxygen was replaced by nitrogen the Golden Bowl seeds showed a greater power of germination and greater oxidase activity than did the seeds of variety Dark Red.

Summary

1. The effect of temperature, an excessive supply of oxygen, a limited supply of oxygen, and the absence of oxygen on the catalase and oxidase activity of imbibed seeds of two varieties of Hibiscus, or rose mallow, was studied.

2. In all seeds capable of germination the catalase activity follows a very definite course. In the earliest stages of germination the catalase activity is depressed but soon increases rapidly as germination progresses. The oxidase content shows some fluctuation at first, then increases according to the rate of germination.

3. Temperature plays an important rôle in the germination of the seeds and in the activity of the enzymes. At low temperatures (8° C.) germination is slowed up as is catalase and oxidase activity. Medium temperatures (20°–27° C.) favor germination and likewise favor increased activity of oxidase and catalase. Temperatures above 40° C. inhibit germination of the seeds; correspondingly the enzymes not only fail to increase but decrease very rapidly.

4. An excess or adequate supply of oxygen favors germination of the seeds and also favors an increase in the activity of oxidase and catalase. When the supply is limited or inadequate there is a tendency for germination to be retarded or inhibited and this is accompanied by corresponding loss of activity of the enzymes.

5. There is a close relationship between the environmental factors and the activity of the oxidizing enzymes in rose mallow seeds.

The writer wishes to express her gratitude for the helpful suggestions given by Professors C. A. SHULL and S. V. EATON during the course of this investigation.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. APPLEMAN, C. O. Some observations on catalase. Bot. Gaz. **50**: 182–192. 1910.
2. ————. Relation of oxidases and catalases to respiration in plants. Amer. Jour. Bot. **3**: 223–233. 1916.
3. ————. Respiration and catalase activity in sweet corn. Amer. Jour. Bot. **5**: 207–209. 1918.
4. BILETZKY, M. Über die Abhängigkeit der Katalaseaktivität der Pflanzen von den Ernährungs- und Wachstumsbedingungen. Fermentforsch. **13**: 467–498. 1933.
5. BUNZELL, HERBERT H. The measurement of the oxidase content of plant juices. U. S. D. A. Bur. Plant Ind. Bull. 238. 1912.
6. CROCKER, WM. and HARRINGTON, G. T. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality and respiration. Jour. Agr. Res. **15**: 137–174. 1918.
7. DELEANO, N. T. Recherches chimiques sur la germination. Centralbl. Bakt. II, **24**: 130–146. 1909.
8. DIXON, M. Studies of xanthine oxidase. V. The function of catalase. Biochem. Jour. **19**: 507–512. 1925.
9. DOYLE, J. and CONNOR, P. O. Seasonal change in catalase content of conifer leaves. Ann. Bot. **44**: 907–915. 1930.
10. EULER, H. and BLIX, R. Verstürkung der Katalasewirkung in Hefezellen. Zeitschr. physiol. Chem. **105**: 83–114. 1919.
11. EULER, N. v. and JOSEPHSON, K. Über Katalase I. Ann. **452**: 158–181. 1927.
12. FALK, G. K. Chemistry of enzyme actions. Chemical Catalog Co. Inc. N. Y. 1924.
13. HAND, D. B. Peroxidase: A comparison with other iron porphyrin catalysts in cells. Ergebn. Enzymforsch. **2**: 272–281. 1933.
14. HARVEY, R. B. Enzymes of thermal algae. Science n.s. **60**: 481–482. 1924.
15. HEINICKE, A. J. Catalase activity as an indicator of the nutritive condition of fruit tissues. Proc. Amer. Soc. Hort. Sci. **19**: 209–214. 1922.

16. ———————. Seasonal variation in the nutrient condition of apple trees as indicated by catalase activity. Proc. Amer. Soc. Hort. Sci. **25**: 234–239. 1929.
17. Lantz, C. W. Respiration in corn with special reference to catalase. Amer. Jour. Bot. **14**: 85–105. 1927.
18. Loew, O. Catalase. U. S. Dept. Agr. Rept. **68**: 1–27. 1901.
19. Marks, G. W. and Fox, D. L. The inactivation of mussel catalase by oxygen. Jour. Biol. Chem. **103**: 269–283. 1933.
20. McLeod, J. W. and Gordon, J. The problem of intolerance of oxygen by anaerobic bacteria. Jour. Path. & Bact. **26**: 332–343. 1923.
21. Morgulis, S. and Beber, N. Studies on the effect of temperature on the catalase reactions. VI. Heat inactivation of catalase at different hydrogen ion concentrations. Jour. Biol. Chem. **77**: 115–126. 1928.
22. Morinaga, T. Catalase activity and the aerobic and anaerobic germination of rice. Bot. Gaz. **79**: 73–84. 1925.
23. Neller, J. T. Relation of catalase activity to physiological breakdown in Jonathan apples. Plant Physiol. **6**: 183–189. 1931.
24. Pope, M. N. Catalase activity in relation to the growth curve in barley. Jour. Agr. Res. **44**: 343–355. 1932.
25. ———————. Catalase activity and respiration in the leaves of growing barley. Jour. Agr. Res. **45**: 35–40. 1933.
26. Rhine, L. E. Divergence of catalase and respiration in germination. Bot. Gaz. **78**: 46–67. 1924.
27. Stern, K. Über die katalatische Aktivität van Häminkomplexen. 5. Über Katalase. Zeitschr. physiol. Chem. **215**: 35–46. 1933.
28. ———————. Forgesetzte Versuch über synthetische Häminkatalasen. 7. Über Katalase. Zeitschr. physiol. Chem. **219**: 105–114. 1933.
29. Sumner, J. B. and Dounce, A. L. Crystalline catalase. Science n. s. **85**: 366–367. 1937.
30. Tauber, H. and Kleiner, I. S. Chemical nature of catalase. Proc. Soc. Exper. Biol. & Med. **33**: 391–392. 1935.
31. Woolf, B. Recent advances in the study of enzymes. Nature (London) **129**: 928–930. 1932.
32. Zeile, K. and Hellstrom, H. Über die aktive Gruppe der Leberkatalase. Zeitschr. physiol. Chem. **191**: 171–192. 1930.

STUDIES ON THE PROTOPLASMIC NATURE OF STIMULATION AND ANESTHESIA. II[1]

HENRY T. NORTHEN

(WITH FIVE FIGURES)

Introduction

In a recent paper the writer (**12**) has discussed the protoplasmic nature of stimulation and anesthesia. It was believed that normal protoplasm has a grid structure which results from combinations of proteins with lipoids and other cell constituents. In unstimulated protoplasm the proteins are presumably in the form of long chains. Upon stimulation some of the proteins are separated from the grid and change from a chain into a globular form. The separation of the proteins from the grid and their change in shape results in a decrease in the structural viscosity of the protoplasm. All of the stimulating agents which we have studied cause a decrease in the structural viscosity of protoplasm (**12**). Since the cited paper was prepared the effects of other stimulating agents upon protoplasm in cells of Spirogyra have been studied and the data presented will demonstrate that electricity, disease, carbon dioxide, cold, and mechanical impacts cause a decrease in the structural viscosity of protoplasm.

If it be true that the primary effect of stimulating agents is a decrease in the structural viscosity of protoplasm which results from a partial breakdown of the protoplasmic network, then other effects of stimulating agents should result from the breakdown of the normal structure. Hence an attempt will be made to explain some of the secondary effects of stimulation on a protoplasmic basis.

In "dormant" cells stimulants often initiate growth. HEILBRUNN (**4**) states that some stimulating agents will initiate cell division in unfertilized animal eggs and many stimulating agents break rest periods in plants (**8**). How may the change in protoplasmic structure initiate growth? To a large extent the activities of a cell are determined by the protoplasmic pattern. In a dormant cell all requirements for growth may be present but the protoplasmic pattern is not favorable for growth. A stimulating agent breaks down the "dormant" protoplasmic pattern. The opportunity then exists for the network constituents to form a new pattern—one which may favor growth. It should also be mentioned that during mitosis and meiosis some of the cytoplasm becomes oriented and distributed. In animal cells amphiasters develop; in plant cells phragmoplasts. They develop in part from

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 178.

the cytoplasm. The development of such structures necessitates the alteration of the protoplasmic pattern of resting cells so that the network constituents may become oriented to form the mentioned structures. Before rearrangement the original structure must be broken down by an external or internal stimulus; if other factors are present in the cell a pattern differing from the original will then be reconstructed and this new pattern may function differently. The breaking down and reconstruction involve changes in the consistency of protoplasm. Changes in the viscosity of protoplasm during mitosis and meiosis have been observed by HEILBRUNN (3), KOSTOFF (5), and others.

Most stimulating agents initially increase the rate of respiration (6). In unstimulated cells some of the substrate and respiratory enzymes are possibly "tied up" to the protoplasmic network. Upon stimulation, when the network is broken down, the enzymes and substrate may be released and accordingly the rate may be increased. It is believed that the increased respiratory rate furnishes the energy for recovery (usually changing the structure back to normal), an example of the auto-regulatory nature of protoplasm.

Stimulating agents generally increase the permeability rate (4). NORTHEN (11) deduced that the selectively permeable membranes have a structure comparable to that of the main mass of the cytoplasm but perhaps more compact. It may be stated tentatively that upon stimulation the structure is loosened, pores are increased in size, and concomitantly the rate with which certain substances may enter or leave is increased.

Response to stimulation is generally followed by conduction (4). A point stimulation will alter the structure locally; the local alteration will affect adjacent parts and hence will alter them. The newly altered structure will, in turn, affect the contiguous structure and in such manner the impulse may be conducted. This is in accordance with the data of NORTHEN and NORTHEN (10) who have found that the conduction of impulses through cells decreases the elasticity (structural viscosity) of the protoplasm.

Experimental results

EFFECTS OF ELECTRICITY ON THE STRUCTURAL VISCOSITY

NORTHEN and MACVICAR (13) have reported that direct currents initially decrease the elasticity (structural viscosity) of protoplasm in cells of the alga, Spirogyra. At that time, however, several important effects were overlooked because the electrodes were placed too close together and because the filaments were not carefully oriented on the microscope slides. In this part, the effects of a direct current on the structural viscosity of protoplasm in cells near the anode and near the cathode and the effects on cells distant from the electrodes will be described.

In this experiment a species of Spirogyra which had one chloroplast in each cell was used. Platinum electrodes with a diameter of 1.0 mm. were connected through an ammeter and rheostat to a source of current and were then placed 1.4 cm. apart on a strip of wet cotton which was partially immersed in water in a Petri dish. Portions of Spirogyra filaments which were 3.0 cm. long were then placed over the electrodes. In each experiment about 100 filaments were used. After a current of 2 ma. had flowed for the desired time (2, 4, 6, 8, 10, or 12 minutes) the filaments were centrifuged. To test whether the current had decreased the structural viscosity of the protoplasm the filaments were centrifuged with an acceleration of 382.5 × gravity for 90 seconds, a centrifugation which would not displace the chloroplasts in cells of control filaments. To test whether the structural viscosity had been increased a separate group of filaments was centrifuged for 40 seconds with an acceleration of 680 × gravity, an acceleration which would displace the chloroplasts in cells of control filaments. Following centrifugation the regions of the filaments where the chloroplasts were displaced and where they were not displaced were noted. The data are summarized in figure 1. The diagrams represent filaments which were exposed to the

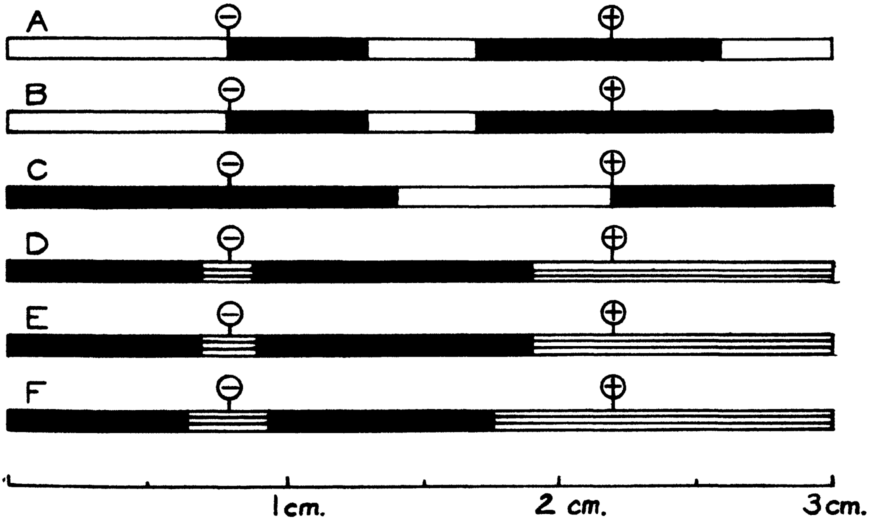


FIG. 1. Effects of a direct current on the structural viscosity of protoplasm.

current for 2 minutes (A), 4 minutes (B), 6 minutes (C), 8 minutes (D), 10 minutes (E), and 12 minutes (F). The white regions are regions where the structural viscosity of the protoplasm remained normal, the black regions are where the structural viscosity was decreased, and the lined regions are those in which the structural viscosity was increased. The structural viscosity was considered decreased if an acceleration of 382.5 × gravity dis-

placed the chloroplasts in cells which were exposed to the current. It will be recalled that such an acceleration would not displace the chloroplasts in control cells. In such cells the chloroplasts were not displaced by an acceleration of 382.5 × gravity because protoplasm has a yield value and the force produced by that acceleration was equal to or less than the yield value. Protoplasm is thus an elastic fluid, a fluid which possesses structural viscosity. NORTHEN (9) has demonstrated that the velocity of chloroplastic movement in cells of Spirogyra can be calculated approximately from the equation, $V = k(c - c_o)$. In the equation, V is the velocity of chloroplastic movement, k is a constant, c is the centrifugal acceleration used, and c_o is the initial starting acceleration (the yield acceleration). Hence for control filaments when an acceleration of 382.5 × gravity was used $V = k(382.5 - 382.5) = 0$ or $V = k(382.5 - > 382.5) = < 0$.

In filaments which were exposed to the current the chloroplasts were moved in some regions by an acceleration of 382.5 × gravity since the current had loosened the protoplasmic network and concomitantly decreased the value of c_o. Hence in such regions $V = k(382.5 - < 382.5) = > 0$. A decrease in the value of c_o has been interpreted as a decrease in the structural viscosity of the protoplasm.

On the other hand, in regions (those lined) exposed to the current and where the chloroplasts could not be moved by an acceleration of 680 × gravity, the value of c_o had been increased to at least 680 × gravity. (In control filaments the value of c_o was less than 680 × gravity but equal to or greater than 382.5 × gravity). An increase in the value of c_o indicates that the structural viscosity of the protoplasm has been increased.

It will be noted, figure 1A, that after two minutes exposure to the current the structural viscosity has been decreased only in the vicinity of the electrodes. Likewise when isolated muscle fibers are stimulated electrically the response may be localized at the electrodes (1).

With exposures of eight or more minutes the structural viscosity is decreased on the cathodal side except in the immediate vicinity of the cathode but increased on the anodal side. It is likely that the protoplasm with increased structural viscosity (which probably indicates that the protoplasmic network has been tightened) is not as sensitive to stimulants as is normal protoplasm and hence may be considered anesthetized. This observation is in accord with the statement by HEILBRUNN (4) that the passage of an electric current may produce a relative insensitivity at the anode.

EFFECT OF DISEASE ON THE STRUCTURAL VISCOSITY

The data presented suggest that the structural viscosity of protoplasm in cells infected with a Chytrid is less than the structural viscosity of protoplasm in normal cells of Spirogyra.

Two species of Spirogyra were found which were infected by Chytrids. The filaments were centrifuged with an acceleration of 382.5 × gravity for one minute, a centrifugation which would not displace the chloroplasts in normal cells. Following centrifugation observations were made to determine whether or not such an acceleration would displace the chloroplasts in infected cells.

The data are summarized in figure 2. Numbers 1 and 2 represent uncen-

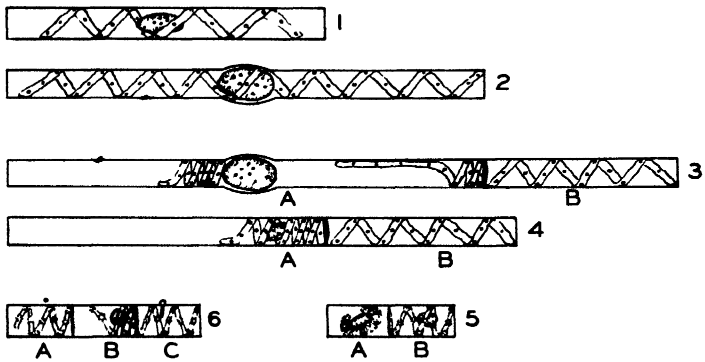


FIG. 2. Effects of disease on the structural viscosity of protoplasm.

trifuged infected cells of Spirogyra 1. Numbers 3 and 4 represent cells from centrifuged filaments. It will be noted nos. 3 and 4 (cells A) that the chloroplasts are displaced in the infected cells and are not displaced in the uninfected cells (cells B). The chloroplasts were probably displaced in the infected cells because the infection caused a decrease in the structural viscosity of the protoplasm. Number 5 represents uncentrifuged cells of Spirogyra 2. In cell A of number 5 the protoplasm has disintegrated and in such cells centrifugation caused no displacement of the cell contents. Number 6 represents centrifuged cells of Spirogyra 2. In the uninfected cell (A) the chloroplast has not been displaced. The chloroplast, however, has been displaced in the infected cell (B), and this indicates that the structural viscosity has been decreased. In cell C infection is just starting and as yet the structural viscosity has not been affected.

EFFECT OF CARBON DIOXIDE ON STRUCTURAL VISCOSITY

Carbon dioxide often acts as a stimulating agent. THORNTON (15) reports that carbon dioxide is effective in breaking the dormancy of potato tubers. LILLIE (7) states that the respiratory center of vertebrates increases its rhythm as carbon dioxide accumulates in the blood. Carbon dioxide may also stimulate the growth of bacteria (16). The data to be presented will demonstrate that the carbonate ion causes a decrease in the structural viscosity of protoplasm in cells of Spirogyra. The decrease in structural

viscosity may result from the separation of network constituents and the subsequent change in shape of protein molecules.

One group of Spirogyra filaments was placed in tap water, another in tap water which was maintained charged with carbon dioxide, another in tap water maintained at a pH of 5.6 (the pH of the charged water) through the addition of hydrochloric acid, and still another group were placed in tap water maintained at pH 5.6 through the addition of acetic acid. After the filaments had been in the solutions the desired time they were centrifuged (for immersion times and centrifugations consult table I). Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced were determined.

The data are summarized in table I and part of the data are expressed

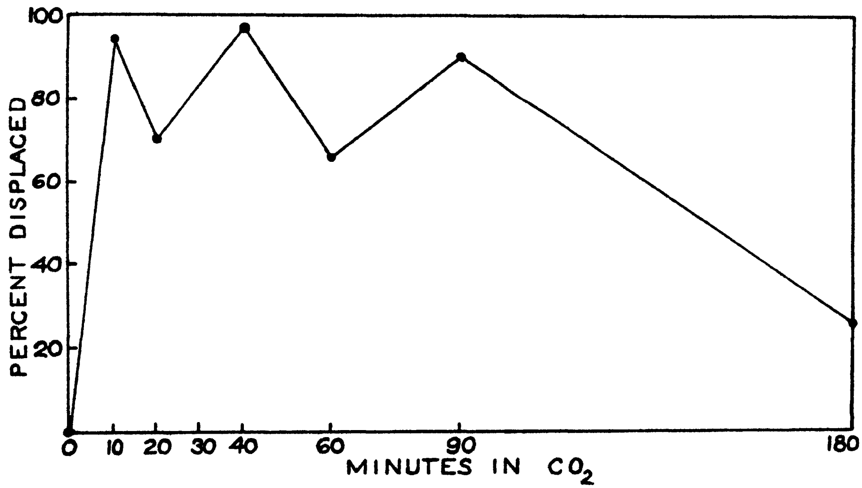


FIG. 3. Effects of tap water charged with carbon dioxide on the structural viscosity of protoplasm. The higher the point on the curve the lower the structural viscosity of the protoplasm.

graphically in figure 3. It will be noted that in those filaments which were exposed to carbon dioxide for 90 minutes or less the chloroplasts were displaced in most cases by an acceleration of 170 × gravity whereas in the control filaments even an acceleration of 680 × gravity was not sufficient to displace the chloroplasts in most of the cells. With exposures to carbon dioxide of 180 and 360 minutes the chloroplasts were displaced in cells of most filaments by an acceleration of 382.5 × gravity. Hence the data indicate that the carbonate ions decidedly decrease the structural viscosity of the protoplasm. The carbonate ions rather than the H ions cause the decrease since acidification of the tap water with hydrochloric or acetic acid did not decidedly affect the structural viscosity. The above experiment was re-

TABLE I

EFFECTS OF CARBON DIOXIDE ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

TIME IM-MERSED	CENTRIFUGATION	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED WHEN IMMERSED IN			
		TAP WATER	TAP WATER AND HCL	TAP WATER AND ACETIC ACID	TAP WATER AND CO_2
min.		%	%	%	%
10	170 × g. for 2 min.	0	4	4	94
20	"	0	0	1	70
40	"	0	0	2	97
60	"	0	0	6	66
90	"	0	0	17	90
180	"	0	0	0	25
180	382.5 × g. for 2 min.	4	3	32	84
360	"	1	2	24	92
360	680 × g. for 2 min.	6			

peated with a different species of Spirogyra with essentially the same results.

In table II data are recorded which indicate that in most filaments recovery from the effects of carbon dioxide occurs in less than five minutes. In this experiment filaments were immersed in water charged with carbon dioxide for five minutes and were then transferred to ordinary tap water. After they had been in the tap water for various periods of time the filaments were centrifuged with an acceleration of 170 × gravity for two minutes. Such a centrifugation did not displace the chloroplasts in cells of control filaments.

TABLE II

TIME REQUIRED FOR THE PROTOPLASM TO RECOVER FROM CARBON DIOXIDE

TIME IN NON-CHARGED WATER	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED
min.	%
1	95
5	17
10	16
15	1
20	3
30	1

EFFECTS OF COLD ON STRUCTURAL VISCOSITY

If organisms are maintained at low temperatures they may become anesthetized (**14**). ETS (**2**) further reports that two types of blocks occur when nerves are locally cooled. In some nerves a block does not occur until after ice has formed (freezing type), whereas in others a block occurs without ice formation. In the latter (non-freezing type) the nerves recover

when the temperature is raised. The cold anesthesia and the non-freezing type of block may be explained on the basis of an increase in the structural viscosity of the protoplasm. NORTHEN (11) has found that the structural viscosity of the protoplasm in cells of Spirogyra increases when filaments are maintained at 1.0° C. which indicates that the protoplasmic network is tightened and accordingly may not break down as readily as the normal network upon stimulation. In other words, the tightened network may be anesthetized. The freezing type of block, one which does not regain conduction when transferred to room temperatures, may be the result of a localized coagulation of the protoplasm. The data presented indicate that temperatures below freezing cause coagulation of protoplasm in Spirogyra.

Not only may low temperature act as an anesthetic but it may also act as a stimulating agent. For example, MAXIMOV (8) states that freezing may be effective in breaking rest periods in plants. HEILBRUNN (4) states that cold may often act as a stimulating agent. Stimulating agents primarily decrease the structural viscosity of protoplasm and anesthetics either increase the structural viscosity or change the pattern from a sensitive to a relatively insensitive one (12). How then may low temperatures act both as anesthetizing and stimulating agents? When an organism is maintained at a low temperature it may become anesthetized but when it is transferred from a low to a higher temperature the stimulating effect may become apparent. In other words, the stimulation may not occur at the low temperature but only after the organism is transferred to a higher temperature. The data to be presented will demonstrate that the structural viscosity of protoplasm in filaments of Spirogyra which were subjected to low temperatures and then transferred to room temperature is less than the structural viscosity of protoplasm in filaments which were not subjected to low temperatures but which were only maintained at room temperatures. Hence cold may be classified as a stimulating agent because it may indirectly cause a decrease in the structural viscosity of protoplasm.

Petri dishes were filled to a depth of about 2 mm. with water at a temperature of 15° C. Filaments of Spirogyra were then placed in the dishes. They were next placed in a refrigerator which was maintained at approximately $-3.0°$ C. After the dishes had been in the refrigerator the desired time they were removed. The filaments were then transferred to water which had a temperature of 20.0° C. The filaments were allowed to remain in this water for various periods of time and they were then centrifuged. Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced (moved to centrifugal end) were determined for experimental and control groups.

The data are summarized in table III and expressed graphically in figure 4.

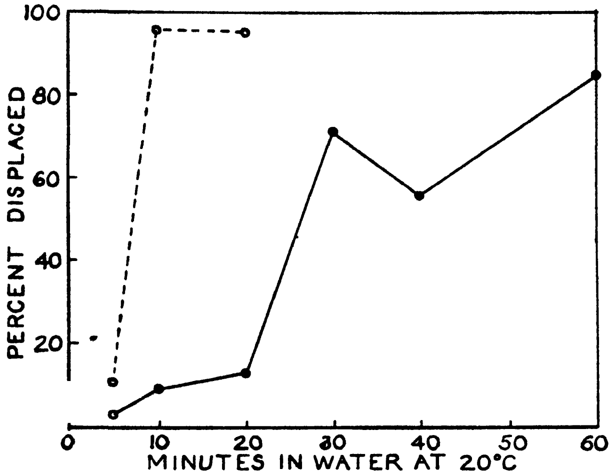


FIG. 4. Effects of previous exposure to cold on the structural viscosity of protoplasm. The filaments were allowed to remain at $-3.0°$ C. for 10 minutes (unbroken line) and 20 minutes (broken line). They were then transferred to water at 20° C. where they were allowed to remain for the indicated times and finally the filaments were centrifuged. The higher the points on the curves the lower the structural viscosity.

It will be noted that following exposures to cold there is a latent period, a period during which the protoplasm of those exposed to cold is apparently like that of those which were not exposed to cold. For those filaments which remained in the refrigerator 20 minutes the latent period was less than 10

TABLE III

EFFECT OF LOW TEMPERATURES ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

TIME FILAMENTS WERE IN REFRIGERATOR	REMARKS	CENTRIFUGATION	PERIOD BETWEEN REMOVAL FROM COLD AND CENTRIFUGATION	FILAMENTS IN WHICH CHLOROPLASTS WERE DISPLACED
min.			*min.*	%
0.0	Control	680 × g. for 0.5 min.		2
0.0	"	1062 × g. for 0.5 min.		18
10.0	Ice just forming over surface of water	680 × g. for 0.5 min.	5.0	3
"	"	"	10.0	9
"	"	"	20.0	13
"	"	"	30.0	71
"	"	"	40.0	56
"	"	"	60.0	84
20.0	Much of water frozen but not directly around filaments	1062 × g. for 0.5 min.	5.0	11
"	"	"	10.0	96
"	"	"	20.0	95

minutes. It will be noted that 10 minutes after the filaments were removed from the cold water the chloroplasts were displaced in the cells of 96 per cent. of the filaments whereas in control filaments a like centrifugation displaced the chloroplasts in only 18 per cent. of the filaments. Hence it is concluded that previous exposure to cold may result in a decrease in the structural viscosity of protoplasm and accordingly cold may be considered a stimulating agent.

During the course of the main experiment several other experiments were performed and these will be discussed. For these experiments a different species of Spirogyra was used. In this species an acceleration of 382.5 × gravity displaced the chloroplasts in the cells of most untreated filaments. One group of filaments was allowed to remain in 0.2 molal sucrose for six hours. They were then transferred to water and placed in a refrigerator maintained at – 3.0° C. Another group of filaments, which had not previously been in sucrose, were likewise placed in the refrigerator. After one hour all of the water in the Petri dishes was frozen. The dishes were then removed and the ice was allowed to thaw. The filaments were then transferred to water at 20° C. and remained at that temperature for ½ hour, 1 hour, 2 hours, and 4 hours, respectively. Finally the filaments were centrifuged with an acceleration of 382.5 × gravity. The acceleration would not displace the chloroplasts in those cells which had previously been in sucrose nor in those cells which had not previously been in sucrose. In both instances the protoplasts had shrunken from the cell walls (figures 5A and 5B). The observations indicate that the freezing had coagulated the protoplasm and that previous immersion in sucrose did not retard the coagulation.

It is likely, however, that the coagulation was not the result of the withdrawal of water and the concomitant increase in the concentration of electrolytes since withdrawal of water by hypertonic sucrose solutions does not cause coagulation (**12**). At the time the freezing experiments were performed filaments of Spirogyra were plasmolyzed in 0.8 molal sucrose. After the filaments had been in the solution for the desired time they were centrifuged with an acceleration of 382.5 × gravity. After the filaments had been in the sucrose solution for ½ hour, 1 hour, 2 hours, or 4 hours the protoplasm was not coagulated as evidenced by the fact that the centrifugation displaced the chloroplasts in most cells. Cells which were allowed to remain in the sucrose solution for 2 hours are diagrammed in figures 5C and 5D. It will be noted that the chloroplasts have been moved by the centrifugal acceleration.

Effects of mechanical impacts on the structural viscosity of protoplasm

The data presented will show that mechanical impacts decrease the struc-

tural viscosity of protoplasm in cells of Spirogyra, that recovery usually occurs in less than 10 minutes, and that stimuli are additive.

Filaments of Spirogyra were placed in water on a slide and were covered with a cover glass. A glass cylinder which surrounded a steel upright was then allowed to fall on the filaments. Following the impulse the filaments were centrifuged and the percentages of filaments in the cells of which the chloroplasts were displaced were determined.

The effects of a 3.20 gram weight dropping distances of 10, 15, 20, 25, and 30 cm. on the structural viscosity of protoplasm are recorded in table IV. In all instances the filaments were centrifuged with an acceleration of 245 × gravity for 2 minutes.

TABLE IV

EFFECTS OF MECHANICAL IMPACTS ON THE STRUCTURAL VISCOSITY OF PROTOPLASM

IMPULSE	FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED
cgs.	%
000.0	1
448.0	8
548.5	35
633.6	32
708.4	51
775.3	72

FIG. 5. A and B represent centrifuged cells which remained in 0.2 molal sucrose for six hours before being placed in a refrigerator at −3.0° C. It will be noted that the chloroplasts have not been moved by the centrifugal acceleration. C and D represent cells which remained in 0.8 molal sucrose for two hours prior to centrifugation. It will be noted that the centrifugation has displaced the chloroplasts in the protoplasts.

It will be noted that in filaments which were subjected to mechanical impacts the chloroplasts were displaced in greater percentages of filaments than in the controls. This indicates that mechanical impacts decrease the structural viscosity of protoplasm probably through loosening the protoplasmic network.

TIME FOR RECOVERY FROM IMPACTS.—Filaments of Spirogyra were subjected to an impulse of 775.3 cgs. After the impulse the filaments were allowed to remain in water for various periods of time before centrifugation. The results are recorded in table V.

At the time experiment no. 1 was performed more than eight minutes were required for complete recovery whereas at the time no. 2 was performed

TABLE V

TIME FOR RECOVERY FROM MECHANICAL IMPACTS

EXPERIMENT NUMBER	CENTRIFUGATION	INTERVAL BETWEEN IMPACT AND CENTRIFUGATION	FILAMENTS IN CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED IN	
			EXPERIMENTAL GROUPS	CONTROL GROUPS
		sec.	%	%
1	245 × g. for 2 min.	15	90	
	"	120	75	42
	"	240	75	
	"	480	54	
2	170 × g. for 2 min.	15	40	
	"	60	26	3
	"	120	4	

recovery occurred in about two minutes as evidenced by the fact that in the controls the chloroplasts were displaced in 3 per cent. of the filaments and in the experimental filaments in 4 per cent. The difference is not considered significant.

SUMMATION OF MECHANICAL STIMULI.—Filaments of Spirogyra were given one mechanical impact and then after various periods of time had elapsed they were given a second impact. To obtain intervals of less than one-half second two weights were dropped simultaneously from different heights. Following the second impact the filaments were centrifuged for two minutes with an acceleration of 170 × gravity.

The data are summarized in table VI.

The data in table VI indicate that mechanical impacts are additive. In experiment 1 the chloroplasts were displaced in 19 per cent. of the filaments which were subjected to a single impulse of 775.3 cgs. and in 17 per cent. of the filaments which were subjected to an impulse of 678.4 cgs. However, if an impulse of 775.3 cgs. were followed a second later by an impulse of 678.4 cgs. the chloroplasts were displaced by the centrifugation in 80 per cent. of the filaments. Likewise in experiments 2 and 3 it will be observed that impulses are additive. In experiments 2 and 3 a single impulse of 1455 cgs. did not have as great an effect as two separate impulses. For example, in experiment 2 centrifugation displaced the chloroplasts in 48 per cent. of the filaments which were given one impulse of 1455 cgs. but displaced the chloroplasts in 71 per cent. of the filaments which were given an impulse of 775.3 cgs. and then 30 seconds later a second impulse of 678.4 cgs. (total = 1437 cgs.). Apparently following the first impact the protoplasmic network is more sensitive to stimulation than it was originally. In experiment 1 the protoplasm was decidedly hypersensitive 0.045 second

TABLE VI

ADDITION OF MECHANICAL STIMULI

EXPERIMENT NUMBER	IMPULSE OF FIRST IMPACT	IMPULSE OF SECOND IMPACT	TIME BETWEEN IMPULSES	FILAMENTS IN WHICH THE CHLO WERE DISPL
	cgs.	cgs.	sec.	%
1	0.0	0.0		1 (water
	775.3	0.0		19
	678.4	0.0		17
	775.3	701.2	0.008	42
	708.4	678.4	0.022	43
	633.6	678.4	0.045	71
	775.3	678.4	1.0	80
	775.3	678.4	15.0	59
	775.3	678.4	120.0	64
	775.3	678.4	240.0	30
	775.3	678.4	480.0	8
2	0.0	0.0		3 (water control)
	775.3	0.0		41
	678.4	0.0		40
	1455.0	0.0		48
	775.3	701.2	0.008	33
	708.4	678.4	0.022	31
	633.6	678.4	0.045	42
	775.3	678.4	1.0	40
	775.3	678.4	15.0	64
	775.3	678.4	30.0	71
	775.3	678.4	60.0	64
	775.3	678.4	120.0	42
	775.3	678.4	240.0	11
3	0.0	0.0		0 (water control)
	678.4	0.0		4
	1455.0	0.0		46
	775.3	678.4	0.5	81
	775.3	678.4	1.0	66
	775.3	678.4	2.0	90
	775.3	678.4	4.0	12
	775.3	678.4	8.0	40
	775.3	678.4	16.0	15
	775.3	678.4	32.0	24
	775.3	678.4	60.0	32
	775.3	678.4	120.0	18
	775.3	678.4	240.0	16
	775.3	678.4	480.0	9
	775.3	678.4	960.0	7

after the first impulse, in the second 15 seconds after the first impulse, and in the third 0.5 second after the first impulse.

Following the period of hyper-sensitivity there may or may not be a period of hypo-sensitivity. The data for experiments 1 and 2 suggest that 480.0 and 240.0 seconds, respectively, after the first impulse the protoplasm is less sensitive than it was originally. However, in experiment three the protoplasm was more sensitive to stimulation 240 and 480 seconds after the first impact than it was originally.

Summation of ether stimulation and mechanical impacts.—In this experiment filaments of Spirogyra were immersed in 0.3 per cent. ether, which is far less than the anesthetic concentration, for various periods of time. Some of the filaments were then subjected to an impulse of 678.4 cgs. Following the impulse the filaments were centrifuged. Filaments which had received only the ether treatment, filaments which had been given only an impulse, and normal filaments were likewise centrifuged. The results are tabulated in table VII.

TABLE VII

Summation of ether stimulation and mechanical impacts

Centrifugation	Immersion in ether	Filaments in the cells of which the chloroplasts were displaced when			
		Immersed in 0.3 per cent. ether	Immersed in 0.3 per cent. ether and then given impulse	Water only	Impulse only
	min.	%	%	%	%
$170 \times g$. for 2 min.	6	6	52		
"	10	8	95	2	17
"	20	3	3		
"	40	3	6		
$245 \times g$. for 2 min.	6	47			
"	10	40		23	62
"	20	53			
"	40	28	64		

Initially 0.3 per cent. ether causes a slight decrease in the structural viscosity of protoplasm. This is clearly indicated in the filaments which were centrifuged with an acceleration of $245 \times$ gravity. Here it will be noted that the chloroplasts are displaced in greater percentages of filaments than in the water controls. With both accelerations mechanical impacts cause a decrease in the structural viscosity of the protoplasm as evidenced by higher percentages of displacements than in the water controls. The filaments which were centrifuged with an acceleration of $170 \times$ gravity demonstrate that there is a summation of stimuli when the filaments were immersed for 6 or 10 minutes in the dilute ether. For example, the chloroplasts were displaced in 8 per cent. of the filaments which were subjected to 0.3 per cent. ether for 10 minutes and were displaced in 17 per cent. of the filaments which were subjected to the impulse but were displaced in 95 per cent. of the filaments which were immersed for 10 minutes in ether prior to the impulse.

Summary

A current of 2 ma. acting for 2 minutes caused a decrease in the structural viscosity of Spirogyra protoplasm in the vicinity of the electrodes.

With durations up to six minutes the decrease in structural viscosity spread to other cells. After more than 6 minutes exposure to the current the structural viscosity increased on the anodal side of the filaments and in a small region near the cathode and decreased in regions adjacent to the region of increased structural viscosity near the cathode. In this and subsequent experiments the structural viscosity was considered decreased if an acceleration which would not displace the chloroplasts in cells of normal filaments displaced the chloroplasts in cells of experimental filaments. The structural viscosity was considered increased if a centrifugal acceleration which displaced the chloroplasts in cells of control filaments would not displace the chloroplasts in experimental cells.

Disease, carbon dioxide, previous exposure to cold, and mechanical impacts caused a decrease in the structural viscosity of the protoplasm in cells of Spirogyra. In most filaments recovery from the effects of carbon dioxide and mechanical impacts occurred in less than 10 minutes.

The effects of two successive mechanical stimuli were additive. Stimulation by dilute ether and mechanical impacts were also additive.

THE UNIVERSITY OF WYOMING
LARAMIE, WYOMING

LITERATURE CITED

1. BROWN, D. E. S. and SICHEL, F. J. M. The isometric contraction of isolated muscle fibers. Jour. Cell. and Comp. Physiol., **8**: 315–328. 1936.
2. ETS, H. N. The effect of potassium on the cold blocking of spider crab nerve. Jour. Cell. and Comp. Physiol., **8**: 101–108. 1936.
3. HEILBRUNN, L. V. The colloid chemistry of protoplasm. Berlin. 1928.
4. ——————. An outline of general physiology. Philadelphia. 1937.
5. KOSTOFF, D. Protoplasmic viscosity of dividing cells in floral buds of tobacco. Protoplasma **11**: 177–183. 1930.
6. KOSTYCHEV, S. Plant respiration. Philadelphia. 1927.
7. LILLIE, R. S. Protoplasmic action and nervous action. Chicago. 1932.
8. MAXIMOV, N. A. Plant physiology. New York. 1938.
9. NORTHEN, H. T. Studies of protoplasmic structure in Spirogyra. I. Elasticity. Protoplasma **31**: 1–8. 1938.
10. —————— and NORTHEN, R. T. Studies of protoplasmic structure in Spirogyra. II. Alterations of protoplasmic elasticity. Protoplasma **31**: 9–19. 1938.
11. ——————. Studies of protoplasmic structure in Spirogyra. IV. Effects of temperature on protoplasmic elasticity. Bot. Gaz. **100**: 619–626. 1939.
12. ——————. Studies on the protoplasmic nature of stimulation and anesthesia. Cytologia **10**: 105–112. 1939.

13. ——————— and MacVicar, R. Studies of protoplasmic structure in Spirogyra. VI. Effects of sound and electricity on protoplasmic elasticity. Cytologia **10**: 18–22. 1939.
14. Parker, G. H. General anesthesia by chilling. Science n. s. **90**: 63. 1939.
15. Thornton, N. C. Carbon dioxide storage. XIII. Relationship of oxygen to CO_2 in breaking dormancy of potato tubers. Contr. Boyce Thompson Inst. **10**: 201–204. 1939.
16. Valley, G. and Rettger, L. F. The influence of carbon dioxide on bacteria. Jour. Bact. **14**: 101–137. 1927.

PHYSIOLOGICAL FACTORS OPERATIVE IN ICE–SHEET INJURY OF ALFALFA[1]

V. G. SPRAGUE AND L. F. GRABER

(WITH FIVE FIGURES)

Introduction

Ice-sheets that contact the surface parts of herbaceous plants for extended periods are among the most injurious of the climatic hazards of many over-wintering crops. Under field conditions they occur primarily in low spots which are not well drained. They may, however, cover large areas regardless of topography because sleet storms prevail in latitudes between those regions which are snow-covered for the major portion of the winter and those which, for the most part, are bare.

Knowledge of the nature of ice-sheet injury is limited, and the work reported here is quite preliminary. It is an attempt to ascertain under controlled conditions some of the basic physiological factors which cause this type of winter injury. It has been suggested that killing by ice-sheets is due to smothering. The exact meaning or interpretation which that word was intended to convey is not clear. BUGAEVSKII and ZITNIKOVA (1) have observed that winter wheat plants beneath an ice crust at $-1°$ C. to $-9°$ C. began to decrease in vigor after the twenty-third day and in fifty-four days all were dead. They suggest a lack of oxygen as a possible cause of death. On the other hand, smothering might be construed to mean the absence of a free diffusion of substances from the organism into the pore spaces of the soil and into the air or conversely, from the air to the organism. Whatever the interpretation of the term may be, efforts to break the ice cover and to eliminate its sealing effects by mechanical means such as disking and other types of scarification, have not proven effective in reducing or preventing ice-sheet injury under field conditions. In 1922, GRABER scarified alternate areas of an ice sheet on an alfalfa field in Wisconsin with a weighted disk. Although the scarification occurred within four days after the formation of the ice-sheet the alfalfa was badly killed whether the ice was broken or allowed to remain intact. He further observed that during the first three months of 1937, a sheet of ice, frozen tightly to the soil surface, covered most of the southeastern quarter of Wisconsin. In desperation, many farmers scarified this ice cover with disks and other farm implements. In no case was such treatment reported as being helpful and the losses from winter-killing of alfalfa that year were in excess of 200,000 acres. Only where an interven-

[1] Contribution no. 151 from the Department of Agronomy, Wisconsin Agricultural Experiment Station, Madison, Wis. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

ing layer of snow prevailed between the ice and the soil, did the alfalfa escape injury.

Experiment I

Methods and procedure

Experiments were begun in the fall and early winter of 1934 to study some of the physiological factors causing ice-sheet injury of alfalfa (*Medicago sativa*). The winter-hardy Grimm alfalfa used in this experiment was sown on the University Farm at Madison, May 15, 1934. Although the plants grew vigorously, the top growth was not cut and the plants entered the dormant winter condition with a high storage of organic reserves. By December 22, 1934, the soil was frozen to a depth of two inches and the plants were well hardened. On this date, the plants were dug from the field and were hurriedly washed with cold water. The tops were removed 2 or 3 inches above the crown, and the roots (with crowns) were stored in damp towels at $-8°$ to $-10°$ C. until needed.

On January 5, 1935, the dormant plants were taken from the cold room to the laboratory and arranged into samples of 10 roots each, selecting as nearly uniform plants as possible. These were trimmed to 10 cm. from the cotyledonary node downward while the crown was trimmed to 2 cm. from the cotyledonary node upward. Twenty-six samples of 10 plants each were placed in twenty-six large Pyrex test tubes. Twelve were stoppered with rubber stoppers and had air as the surrounding medium; twelve more were filled with nitrogen, by displacement of water, and stoppered; the remaining two tubes were provided with an inlet and outlet tube through which air was introduced continuously at a constant rate.

On the above date, twelve additional samples of ten plants each were frozen in solid cakes of ice. This was effected by making heavy collodion bags in a $1\frac{1}{2}'' \times 8''$ test tube and, while filled with warm water, varnishing

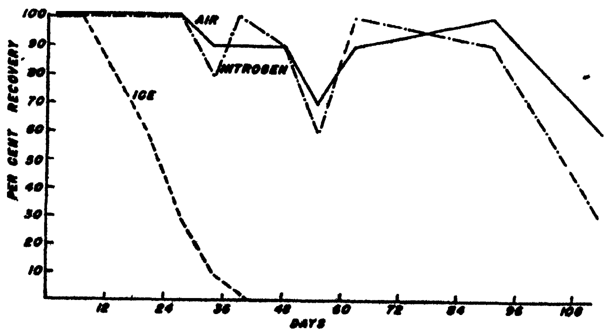


Fig. 1. Percentages of survival after hardened Grimm alfalfa plants were stored in ice and stagnant gaseous media at $-3.5°$ C. for 113 days. Survival values were ascertained by exposure of the plants to favorable conditions for growth in the greenhouse for 3 weeks. Experiment I. Series of January 7 to May 8, 1935.

them with two coats of a high grade water-spar varnish which was impermeable to the alcohol in the freezing bath. A sample of ten plants was placed in each of twelve such bags. The roots were evenly spaced within the bag, covered with tap water, and a cork securely fastened into the top. These samples and those in stoppered test tubes were then suspended in an alcohol-water bath at $-3.5°$ C. They were maintained at this temperature until removed at intervals of several days, when they were thawed at 1° to 2° C. After thawing they were transplanted into sand and placed in the greenhouse for three weeks when determinations of survival and measurements of top growth were made. These are presented in table I and figures 1 and 2.

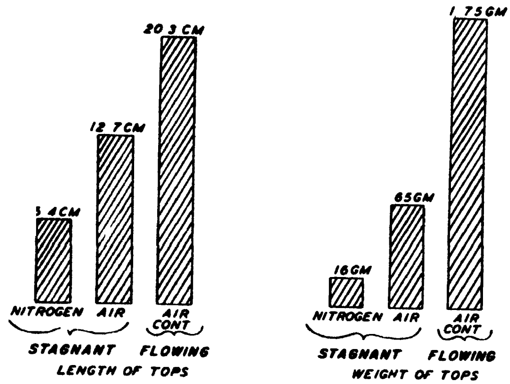


FIG. 2. Vigor of alfalfa plants expressed by the average length of stems and dry weight of top growth after 3 weeks of exposure to favorable growth conditions in the greenhouse. Such plants had been subjected to storage in test tubes of gaseous media for 113 days at $-3.5°$ C. Experiment I. Series of January 7 to May 3, 1935.

VIGOR AND SURVIVAL AS INFLUENCED BY STORAGE IN VARIOUS MEDIA

With only 7 days of storage at $-3.5°$ C., all the plants survived regardless of the surrounding media. With greater duration of the treatments, the plants in ice were first to decline in vigor and percentage of survival, followed somewhat later by the samples stored in stagnant nitrogen, and, finally, by the samples stored in stagnant air. The plants maintained in ice for more than 7 days declined very rapidly and at the end of 41 days all were dead. The plants maintained in stagnant nitrogen for more than 27 days were weakened and recovery was slow, but 90 per cent. were still alive at 91 days and 30 per cent. at 113 days. The plants kept in stoppered tubes of air had higher survival and stronger recovery than those maintained in nitrogen while those in flowing air (table I) survived 100 per cent. and showed only slightly reduced vigor even after 113 days of storage. The latter were much more vigorous than those stored in the stoppered tubes of air or nitrogen. These results are illustrated in figure 2.

TABLE I

GROWTH RECOVERY OF HARDENED ALFALFA PLANTS AFTER HAVING BEEN STORED IN VARIOUS MEDIA FOR VARYING PERIODS AT – 3.5° C. EXPERIMENT I. SERIES OF JANUARY 7, 1935, TO MAY 3, 1935

DAYS STORED	PERCENTAGE SURVIVAL WHEN STORED IN			
	ICE	N_2 (STAGNANT)*	AIR (STAGNANT)*	AIR (FLOWING)
	%	%	%	%
2	100	100	100	
7	100	100	100	
20	60 (weak)	100	100	
27	30 (weak)	100	100	
34	10 (weak)	80 (weak)	90	
41	0	100 (weak)	90	
48	0	90 (weak)	90	
55	0	60 (weak)	70	
62	0	100 (weak)	90	
91	0	90 (weak)	100	
113	0	30 (weak)	60 (weak)	100 (strong)

* Test tubes containing plants were stoppered.

Experiment II

METHODS AND PROCEDURE

The plants used in this experiment were selected from a lot of very winter-hardy Turkestan alfalfa. The seed was sown on the University Farm, July 25, 1935, in a rich loam soil and with favorable weather the plants attained a good size by the time fall hardening began. On November 30, 1935, several thousand plants were dug from the field. The plants with very small and extremely large roots were discarded and 1200 fairly uniform plants were thus obtained. Of these, 200 were reset in each of six tin boxes and buried in the field to ground level. This provided an abundant and efficient source of hardened material without entailing the difficulties of removing individual plants from frozen soil. By using this method of hardening and storing in the field, the plants could easily be taken at intervals during the winter to a cool greenhouse for gradual thawing and removal from the containers. After thawing, the plants were taken to the laboratory, hurriedly washed in cold tap water, trimmed to root lengths of 10 cm. and to crown lengths of 2 cm. as before. These were grouped into uniform samples of ten plants each.

In this experiment the hardened plants were exposed to a temperature of – 4° C. A continuous flow of air was drawn through the test tubes containing one series of plants; a flow of nitrogen through another, and a flow of carbon dioxide through another. One series was frozen in ice as in Experiment I.

In Experiment I some difficulty was encountered with the plants frozen in ice because the collodion bags coated with varnish became brittle and ex-

tremely fragile when subjected to the freezing temperatures. In some cases this resulted in cracked bags which allowed the alcohol of the bath to enter. These samples were, of course, discarded. To overcome this difficulty, rubber latex tubes approximately $1\frac{1}{2}'' \times 7''$ were obtained and the roots were placed in these. The individual plants were separated within the ice as in the previous experiment. The tubes were filled with cold tap water and the tops of the tubes were closed with a large cork before suspension in an alcohol-water bath at $-4°$ C.

The samples to be frozen in circulating air, nitrogen, and carbon dioxide were placed in $1'' \times 8''$ Pyrex test tubes which were fitted with an inlet tube extending just below the stopper and an outlet tube extending to the bottom of the test tube. This insured a constant flow of the gases through the test tubes without stratification. The test tubes through which each gas was introduced were arranged in series and connected by means of short pieces of rubber tubing. Between the root samples and the gas supplies Erlenmeyer flasks, filled with finely chopped ice to saturate the gas with water vapor, were inserted.

The compressed air line in the laboratory provided the source of air. A pressure regulator was installed to provide a slow but constant flow. The nitrogen was obtained by passing air through two large absorption bottles containing alkaline pyrogallate. It was then washed in water and concentrated sulphuric acid. The carbon dioxide was obtained from dry ice. A 2-pound piece of solid carbon dioxide was wrapped in cheese cloth and placed in a gallon thermos jug fitted with a large one-hole rubber stopper. The jug was placed in the freezing chamber to prevent too rapid evolution of the carbon dioxide. The gas was washed in concentrated sulphuric acid and the pressure and rate of flow regulated automatically.

The tubes containing the plants were properly connected with the sources of gas and placed in the thermostatically regulated alcohol-water bath at $-4°$ C. Samples of all series were removed at intervals of about 7 days. The roots were allowed to thaw at 1° to 2° C. for 10 to 12 hours, after which they were transplanted into quartz sand for recovery in the greenhouse. The extent of injury was measured by the weight of dry matter of tops obtained from the various samples after three weeks of growth. Greenhouse conditions were not entirely uniform for each three weeks' period—varying considerably in the amount of sunlight during the winter months. However, the relative growth of the samples tested in any single three weeks' period was comparable.

Effects of storage in various media in experiment II

The results of this experiment are recorded in table II and are presented in graphic form in figure 3. It is evident that little injury takes place with

TABLE II

RECOVERY OF HARDENED ALFALFA PLANTS AFTER STORAGE IN VARIOUS MEDIA FOR VARYING PERIODS AT −4° C. AS MEASURED BY THE LENGTH AND OVEN-DRY WEIGHT OF TOP GROWTH PRODUCED DURING THREE WEEKS OF RECOVERY IN THE GREENHOUSE. EXPERIMENT II. SERIES OF DECEMBER 14, 1935, TO FEBRUARY 5, 1936

DAYS STORED	ICE		CO_2 FLOWING		AIR FLOWING		N_2 FLOWING	
	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.331		0.440		0.440			
9	0.580		0.590					
17	0.400		0.630				0.625	
23	0.378		0.472					
28	0.211	4.54	0.365	7.65	0.614	11.37	0.442	8.69
35	0.223	1.48	0.270	2.22	0.534	4.88	0.464	4.64
53	0.005	0.52	0.230	4.21	0.343	5.66	0.510	7.66

freezing at −4° C. under any of the conditions of surrounding media not in excess of ten days' duration. The growth for three weeks of the unfrozen check sample of plants before storage was much less than those stored in ice for 10 days and then allowed to recover for three weeks. More optimum light conditions in the greenhouse during the latter three weeks' recovery period may account, in part, for the increase. In any case, it indicates that the factors associated with the duration of the ice, rather than direct effects of the ice itself, are harmful.

In this experiment, as well as in Experiment I, the medium of ice was by far the most injurious. With 53 days of storage in ice nearly all the plants died. The injury curve for the flowing carbon dioxide follows that of ice but the injury was less intense. The plants held in flowing air and

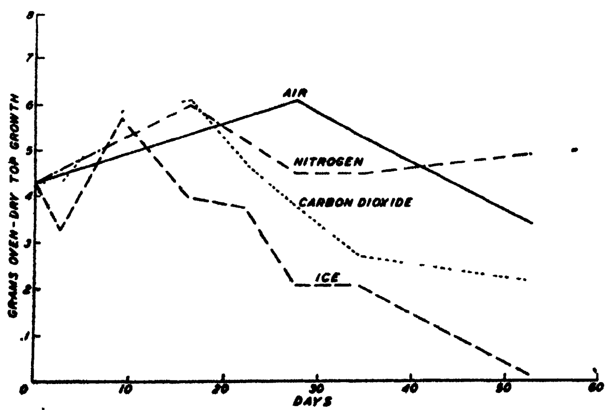


FIG. 3. Weight of oven-dry top growth of hardened Turkestan alfalfa produced in 3 weeks of greenhouse recovery after storage at −4° C. in ice and flowing gases. Experiment II. Series of December 14, 1935, to February 5, 1936.

flowing nitrogen were only slightly injured during this period but at the end of 53 days, the plants in air showed greater injury than those in nitrogen. In all probability this was due to more severe desiccation since the rate of the air flow was greater than that of nitrogen.

The trials of experiment I and II indicate that injury is intensified primarily by the *type* and *duration* of the surrounding media. This appears to be associated with accumulations and increasing concentrations of carbon dioxide and other products of aerobic and anaerobic respiration. The diffusion of carbon dioxide and other respiratory products away from living cells of plants tightly incased in ice, is definitely inhibited by the ice itself and it would appear that, in time, concentrations of considerable magnitude would result in serious injury. None of the surrounding media used in experiments I and II caused such intense and such rapid injury as did ice, and in no case would the diffusion of respiratory products be inhibited more completely. Further investigations of such relationships are incorporated in experiments III and IV.

Experiment III

Methods and procedure

To provide a series frozen at $-4°$ C. for comparison with a series stored in water at 1° C. in experiment IV, a second lot of hardened Turkestan alfalfa plants was dug from the field on January 11, 1936. These were prepared as previously and subjected to $-4°$ C. with various surrounding media similar to those in experiment II, namely, flowing air, flowing nitrogen, flowing carbon dioxide, stagnant nitrogen, and ice.

The recovery of these series as measured by the top growth produced

TABLE III

Recovery of hardened alfalfa plants after having been stored in various media for varying periods at $-4°$ C. as measured by weight and length of the top growth produced during three weeks under greenhouse conditions. Experiment III. Series of January 11, 1936, to March 11, 1936

Days stored	Air flowing: Weight	Air flowing: Length	CO_2 flowing: Weight	CO_2 flowing: Length	N_2 stagnant*: Weight	N_2 stagnant*: Length	Ice: Weight	Ice: Length
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.678	13.61	0.569	10.76			0.437	9.3
10	0.542	11.85	0.423	8.76			0.287	4.28
23	0.488	8.26	0.455	8.70			0.170	2.36
31	0.635	12.08	0.210	4.91	0.325	7.44	0.002	0.9
38	0.510	6.23	0.370	5.37			0.040	0.55
45	0.705	10.34	0.200	7.26	0.260	4.38	0.0	0.0
53	0.540	10.39	0.215	5.41	0.110	2.25	0.0	0.0
60	0.413	4.33	0.010	0.92	0.100	2.20	0.0	0.0

* Test tubes were stoppered.

during three weeks of greenhouse recovery is presented in table III.[2] Figure 4 shows graphically the recovery as measured by oven-dry weight of top

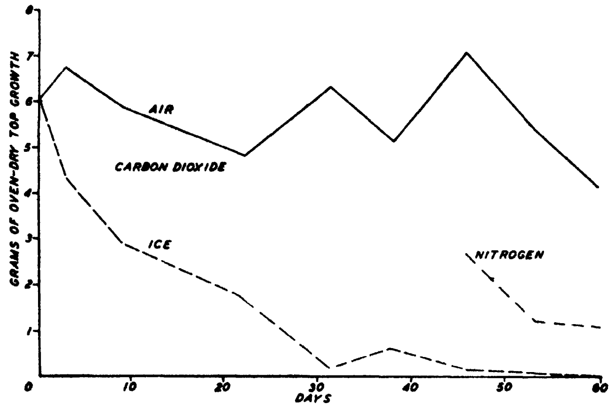


FIG. 4. Weight of oven-dry top growth of hardened Turkestan alfalfa produced in 3 weeks of greenhouse recovery after storage in ice, flowing air, flowing carbon dioxide, and stagnant nitrogen at -4° C. Experiment III. Series of January 11 to March 11, 1936.

growth produced during three weeks of favorable growing conditions in the greenhouse.

In this, as in previous trials, samples of alfalfa plants incased in ice show the greatest injury in the shortest time. Those stored in test tubes of flowing carbon dioxide are next in order but injury was very slight with storage in the medium of flowing air (fig. 4). With 60 days of storage all the plants in ice were dead and nearly all those in flowing carbon dioxide were dead. Those stored in stoppered tubes of nitrogen had slightly better survival but the plants were very much weakened after 60 days of storage.

The photograph (fig. 5) taken after three weeks of greenhouse recovery of the samples of plants removed after 31 days of storage shows a vigorous recovery of the plants frozen in flowing air and flowing nitrogen (both of which would reduce the accumulation of carbon dioxide and other respiratory products), but growth was retarded slightly (and later, severely) for those plants frozen in stoppered tubes of nitrogen where respiratory products could accumulate, and very much for those plants frozen in a stream of carbon dioxide. The plants frozen in ice were either dead or so seriously injured that very little or no top growth was produced.

In these trials and those of experiments I and II, it is probable that with the absence of oxygen in the surrounding gaseous media, the products

[2] Because of circumstances of an accidental character the data on flowing nitrogen is omitted except for results obtained after 31 days of storage which are shown photographically in figure 5.

FIG. 5. Turkestan alfalfa plants after 3 weeks of recovery in the greenhouse. The plants (reading from left to right) had been stored at $-4°$ C. for 31 days (January 11 to February 11, 1936) in flowing air, flowing nitrogen, stoppered test tubes of nitrogen, flowing carbon dioxide, and ice.

of anaerobic respiration become sufficiently concentrated to be toxic. Such harmful concentrations do not occur as rapidly in test tubes which provide for some diffusion as they do with ice which inhibits diffusion from the tissues almost completely.

Experiment IV

Methods and procedure

On January 11, 1936, hardened Turkestan alfalfa roots were obtained from the same field source as those in experiments II and III. They were trimmed as before and arranged in uniform ten-plant samples in Pyrex test tubes. Individual samples were surrounded at 1° C. with the following media: Tap water in stoppered test tubes, tap water through which carbon dioxide was bubbled, tap water through which air was bubbled, stagnant air, and stagnant nitrogen. After various periods of storage, ten-plant samples from each treatment were planted in quartz sand in the greenhouse for recovery.

Results with storage above freezing

The dry weight and length of tops produced during the recovery period following the treatments at 1° C. are presented in table IV and the data are comparable with those in table III. Unfrozen water produced the same detrimental effect as ice and the recovery of the plants stored in water closely

TABLE IV

RECOVERY OF HARDENED ALFALFA PLANTS AFTER STORAGE IN VARIOUS MEDIA FOR VARYING PERIODS AT 1° C. AS MEASURED BY THE WEIGHT AND LENGTH OF TOP GROWTH PRODUCED IN THREE WEEKS UNDER GREENHOUSE CONDITIONS. EXPERIMENT III. SERIES OF JANUARY 11, 1936, TO MARCH 11, 1936

DAYS STORED	IN WATER						NO WATER	
	AIR*		CO_2*		STOPPERED		N_2 STAGNANT	
	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH	WEIGHT	LENGTH
	gm.	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*	*gm.*	*cm.*
3	0.747	14.11	0.679	12.62	0.789	14.86		
10	0.598	14.30	0.471	11.53	0.712	15.23		
23	0.170	4.10	0.01	1.6	0.115	3.04		
31	0.110	2.64	0.0	0.0	0.001	0.2	0.50	11.20
38	0.440	5.13	0.001	0.2	0.125	2.88		
45	0.380	5.19	0.0	0.0	0.001	1.2	0.45	7.03
53	0.120	2.59	0.0	0.0	0.0	0.0	0.220	6.30
60	0.162	3.88	0.0	0.0	0.002	2.0	0.487	7.50

* Bubbled through test tubes of water.

resemble those of plants stored in ice but water saturated with carbon dioxide caused the greatest harm and all of the plants were severely injured in 23 days. Plants in stoppered tubes of water were nearly all dead in 31 days, while those in tubes of water with air bubbling through it were alive and vigorous at the end of 60 days. Plants stored in stagnant nitrogen but without water were healthy and vigorous at the end of 60 days.

In most cases, the injury occurred in the crowns. Often the crowns were dead, while the roots of such plants appeared uninjured. Especially was this true of the plants stored in water saturated with carbon dioxide. Field studies of GRABER (2) in 1922 showed that only the crowns and upper portions of the roots were injured and that the older thickened rhizomes of the crown were often dead, while the young rhizomes diverging from them were alive and apparently not seriously injured. Such plants, of course, eventually died, but the young rhizomes grew until warm weather and desiccation killed them. Combining these observations with those of experiment III, it would seem that ice-sheet injury under field conditions may be due, in part, to accumulations of carbon dioxide where the ice-sheet contacts the plants and that the older rhizomes are more susceptible to such injury. In these experiments, however, only young plants were used. The observations on such plants frozen at $-4°$ C. in ice for 35 days in experiment II and for 23 days in experiment III showed a noticeably higher degree of injury of crowns than of the roots, although in several instances the entire root was killed and decomposed to a pulpy consistency. This suggests that the crowns may be more sensitive to such injury or that the

carbon dioxide and other respiratory products may accumulate there in greater abundance.

The survival of hardened alfalfa plants with ice contacts appears to be related to the time of removal of the plants from their field environment. Plants removed on December 14, 1935, were injured by ice duration to a much less degree than plants given the same treatment after removal from the field on January 11, 1936. This may be of significance but an explanation will become available only with further trials.

Discussion

The authors have not found any literature dealing with the fundamental aspects of injury to alfalfa or other plants from external contacts with ice. While the exact mechanism of this type of cold injury is unknown the results obtained throw some light on some of the factors conditioning ice injury. These experiments were planned to eliminate direct injury from low temperatures so that the influences associated with the media immediately surrounding the plants could be ascertained. Aside from carbon dioxide, none of the surrounding media appeared to be directly injurious.

The interpretation of results obtained in the trials where gaseous and liquid media were confined in test tubes of limited capacity, would be incomplete without an appreciation of the fact that the daily production of carbon dioxide and other respiratory products by dormant plants would gradually increase the concentration of such products in the media and in the plant. The time factor of injury is probably associated with such increasing concentrations of carbon dioxide and other toxic materials of aerobic and anaerobic respiration. More than anything else this may explain the rapid rate of injury sustained by plants frozen in ice. Ice inhibited the removal, escape, and external diffusion of carbon dioxide and other products of aerobic and anaerobic respiration. True, ice excluded oxygen but the exclusion of this element does not seem to be an immediate cause of injury or death. Before the absence of oxygen became a lethal factor, it appears that concentrations of carbon dioxide and other respiratory products resulting from the incasement of the plant in ice, became the lethal factors. Plants stored in stoppered test tubes of nitrogen where oxygen was absent but where some diffusion of carbon dioxide could prevail, were injured less rapidly and much less intensely than plants frozen in ice.

That the removal of carbon dioxide and other respiratory products is associated with factors which greatly reduce the injuries of storage, is shown by the results of all the trials of this investigation. Plants stored in stoppered test tubes of water, were injured as intensely and as rapidly as those incased in ice but plants stored in water through which air was bubbled, were scarcely injured. Plants stored for 113 days at $-3.5°$ C. in stoppered

tubes of air showed decidedly greater injury than those stored in tubes of flowing air. Plants frozen in a constant stream of carbon dioxide showed a degree and rate of injury of a magnitude approaching that occasioned by ice-sheets.

The most rapid injury occurred when plants were stored in water saturated with carbon dioxide. When external accumulations of carbon dioxide were in contact with the plants in water solution, the injury was much greater than external accumulations in the form of gas. Carbon dioxide was the only form of external media used in these trials which appeared to be directly harmful. The rapid injury by carbon dioxide in water solution was observed by FREE (3) in 1917. He reported that buckwheat roots in culture solutions through which carbon dioxide was circulated "sickened and wilted within a few hours and died within a few days."

Summary

The injuries sustained by hardened alfalfa plants frozen and maintained in ice at harmless temperatures, appear to be due to internal accumulations of the toxic products of aerobic and anaerobic respiration. Ice, contacting the root and crown, inhibits the diffusion of carbon dioxide and concentrations of it and other respiratory products may increase rapidly, becoming highly injurious and ultimately lethal.

The degree and rate of injury of hardened alfalfa plants during various periods of storage was, in all cases, closely associated with conditions of the surrounding media which affected the removal of the respiratory product, carbon dioxide. Dormant alfalfa plants stored at non-injurious temperatures of $-3.5°$ C., $-4°$ C., and 1° C. for varying periods in test tubes of external media which removed carbon dioxide or, at least, prevented its accumulation, such as flowing air, and air bubbled through water, and flowing nitrogen, had a much higher survival and sustained much less injury and a much lower rate of injury than those confined for similar periods in ice and in test tubes of surrounding media, such as still water, still nitrogen, flowing carbon dioxide, and especially carbon dioxide bubbled through water.

The formation of ice about and in contact with the plant did not result in direct injury. Only after seven to ten days' duration of the ice were injurious effects manifested but after twenty days a high rate of mortality prevailed. Plants immersed in test tubes of water at 1° C. were injured at approximately the same rate and degree as those frozen in ice at $-4°$ C. Where carbon dioxide was bubbled through water, however, such saturation reduced the survival much more rapidly and injuries were more intense than those sustained from ice contacts. Carbon dioxide was the only one, of the several gaseous media used, that appeared to be directly toxic to

plants and it was much more injurious when in solution than in the gaseous forms.

The exclusion of oxygen did not appear to be an initial cause of injury or death but may be associated with injuries resulting from the accumulations of the products of anaerobic respiration.

The writers wish to express their appreciation to Dr. B. M. DUGGAR and Dr. W. E. TOTTINGHAM for their helpful suggestions during the course of these experiments.

UNIVERSITY OF WISCONSIN

LITERATURE CITED

1. BUGAEVSKII, M. F., and ZITNIKOVA, K. S. Choking winter wheat plants under an ice crust. (Trans. Title), Zbirn. Prac. (Rob.) Agrofiziol. **2**: 103–105. 1936.
2. GRABER, L. F. Twenty-first annual report of the Wisconsin Agricultural Experiment Station. p. 40. 1923.
3. FREE, E. E. The effect of aeration of the growth of buckwheat in water culture. Johns Hopkins Cir. 293. p. 198. 1917.

THE RELATIVE RATES OF DESTRUCTION OF PECTIN IN MACERATES OF VARIOUS CITRUS FRUITS[1]

M. A. JOSLYN AND A. SEDKY

(WITH NINE FIGURES)

Introduction

The natural pectins are believed to consist of long, open, unbranched chains of galacturonic acid with an occasional arabinose and galactose molecule. The free carboxyl groups of the galacturonic acid are partially or wholly esterified with methyl alcohol, and an occasional hydroxyl group is esterified with acetic acid [BONNER (4), BRANFOOT (5), FREUDENBERG (7), GADDUM (8), KERTESZ (13), MYERS and BAKER (16), NORMAN (18, 19), RIPA (22)]. The differences found among the natural pectins are believed to arise from differences in the extent of esterification, and in the extent of polymerization. The view that pectins originate phytochemically by the progressive oxidation of the sixth carbon atoms of the galactose residues occurring in galactans, followed by decarboxylation has been recently questioned by the finding of HIRST and JONES (10) that the araban found in close association with the pectic acid of the peanut is composed entirely of the arabofuranose residues instead of the expected arabopyranose residues.

On the assumption that the pectin molecule consists of galacturonic acid, galactose, arabinose, acetyl, and methoxyl units the pectic enzymes should number at least seven specific enzymes acting upon the possible linkages between these units. In addition we would expect the presence of enzymes of initial attack differentiated by their ability to attack pectins of different degrees of polymerization. Accepting "protopectinase" as such an enzyme, there are at present known but four specific enzymes (13) of which pectin methoxylase has been most widely studied.

The complexity of the pectins, the diversity of the enzymes acting upon them and the difficulty of obtaining either pure pectic enzymes or substrates of definite constitution and configuration have complicated this field. It has been only recently that systematic studies on fairly well defined substrates were begun by EHRLICH (6) and independently by KERTESZ (13). The separation of the various components of the pectinase complex, their purification and investigation is yet to be undertaken. ROTHSCHILD (23) pointed out in this connection the possibility of separating "pectolase" from "pectase" by making use of the different rates at which these are destroyed in acid and alkaline solutions.

[1] Presented at the 23rd annual meeting of the Pacific Division, A.A.A.S., Stanford University, June 29, 1939.

Although some investigations were made by GADDUM (6) on the effect of maturity and fruit type on the chemical composition and physical properties of the pectins extracted from the albedo and pulp of citrus fruits, by HARVEY and RYGG (9) and by RYGG and HARVEY (24) on changes occurring in the pectic constituents of the peel of citrus fruit, no investigations on the nature and relative activity of citrus pectic enzymes have been reported. The citrus pectins are known to differ from each other and from those of other fruits in chemical composition and degree of polymerization. SVEDBERG and GRALEN (27) reported that the pectins from apples, pears and plums have approximately the same molecular weight while that from oranges was of definitely higher molecular weight (40,000–50,000). NORRIS (20) found that the pectic substances in oranges were less methoxylated than those found in lemons by NORMAN (17). Differences between commercial apple and citrus pectins were reported by STUEWER *et al.* (26). It has been shown, particularly by HIRST and co-workers at Birmingham (2, 11, 12), that the araban content of pectins may be reduced to negligible amounts by special methods of preparation so that arabinose need no longer be considered as an essential constituent of pectin. The precise nature of the galactan portion is not known (*cf.* NORRIS (21)) although strong evidence has been adduced to show that the fundamental feature of the chemical structure of both the pectic acid from strawberries and from citrus fruits consists of a chain of pyranose d-galacturonic acid residues linked together through positions 1 to 4, BEAVEN and JONES (3), SMITH (25). LEVENE *et al.* (15) believe the linkage is through the fifth carbon atom. This change in constitution of pectin reduces the number of enzymes in the pectinase complex. There is particular need at present for purification of the components of the pectinase complex as an aid to the study of the physiology and chemistry of pectins.

To obtain some information on the activity of the pectic enzymes present in citrus fruits, an investigation was made of the rate of decomposition of pectin in macerated tissues under various conditions. In preliminary investigations it was found that pectin destruction in oranges occurred at a very different rate from that in apple or cucumber tissues (fig. 1). In apple

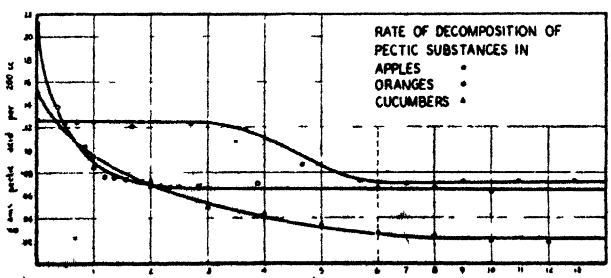


FIG. 1. Rate of decomposition of pectic substances in apples, oranges, and cucumbers.

the total pectin remained essentially constant for four days, then rapidly decreased to about two-thirds of its original value and remained at that level. In oranges the total pectin rapidly decreased to about one-third of its original value in 24 hours and then remained essentially constant, while the pectin content of cucumbers decreased rapidly and continually, reaching a very low level in about 6 days. The constant level in oranges and apples may be ascribed either to the presence of residual pectin unattacked by the enzymes present, or to the decay of the enzymes.

Materials and methods

The data shown in the graphs of figure 1 were obtained with Newton Pippin apples removed from cold storage in July 1937, fresh Valencia oranges and cucumbers. The macerates had the following initial pH values: 3.8, 4.5, and 5.8, respectively and a final pH of 3.5, 4.1, and 4,3, respectively.

The rates of destruction of pectin in orange, grapefruit, and lemon macerates were determined at their natural pH and in macerates adjusted to pH 2.5, 3.5, and 4.5, respectively and these data are presented here.

Preparation of samples

Valencia oranges, Marsh grapefruit, and Eureka lemons purchased early in the fall on the market, were peeled, the peel and pulp group ground separately, mixed together and 0.3 per cent. of sodium benzoate added as preservative. The time required for the preparation of the ground fruit was approximately two hours. The pH was then determined and, four hours after this determination, the entire amount of each fruit was divided into three lots; one of these was left at its original pH (3.5 for grapefruit and oranges and 2.5 for lemons) and the other two lots adjusted to give the range of 2.5, 3.5, and 4.5. Concentrated citric acid was added to obtain a lower pH and sodium hydroxide to obtain a higher pH. Immediately, and at definite intervals thereafter 50-gram samples were withdrawn after thorough mixing and boiled with 400 ml. of water for one hour to inactivate the enzymes and extract the pectin present, water being added periodically to maintain a constant volume. Extracts were then made up to 500 ml. and filtered. The appearance of the extracts shown in figure 2 illustrates the marked changes in pectin content (cloudy samples containing most).

The pectins present in 200-ml. aliquots of the filtrate were precipitated and determined as pectic acid (digalacturonic acid) by the Wichman-Chernoff procedure (1). On the assumption that the mineral content in all the samples withdrawn from a given lot of fruit is constant, the ignition of the pectic acid precipitate was omitted. In three of the series of tests the alcohol precipitate was determined, also, as a measure of gums, pectins, etc.

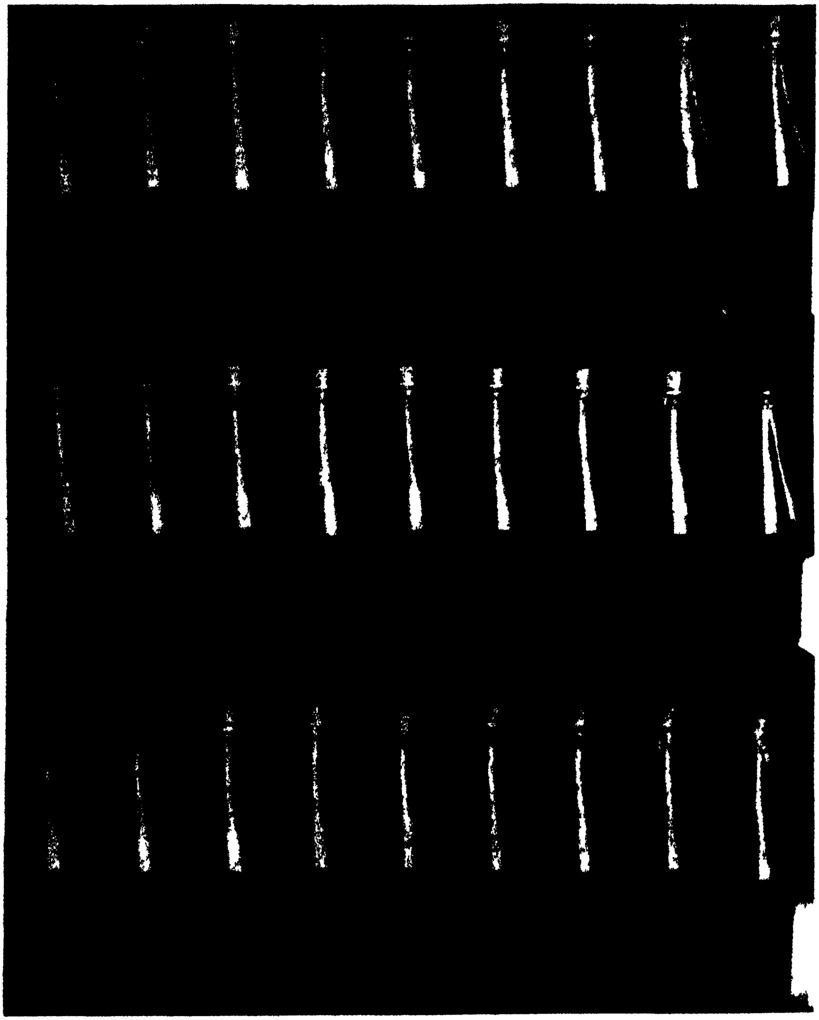


Fig. 2. Appearance of pectin extracts from crushed oranges, grapefruit, and lemons held at their natural pH value.

In the course of these experiments, the vacuum oven at 70° C. and the hot air oven at 100° C. were compared in the drying of pectic acid precipitate in some determinations, using extracts from oranges. The period of drying in the vacuum oven was approximately 24 hours, while that for the hot air oven was 8 hours. A comparison between the values obtained is given in table I.

It is evident that there is but a slight difference between the two methods of drying. The difference can be neglected, particularly in studies which

TABLE I

Comparison of drying by vacuum oven and by hot air oven

Time of storage before determination	Vacuum oven	Hot air oven
	Pectic acid per 200 ml.	Pectic acid per 200 ml.
hr.	*gm.*	*gm.*
0	0.214	0.213
4	0.185	0.183
8	0.157	0.156
12	0.127	0.127
14	0.119	0.118
22	0.102	0.102

are comparative in purpose rather than quantitative, therefore it is possible to use the hot air oven for drying pectic acid precipitates without seriously affecting the results.

Changes in pH value

Determinations of the pH values of the fruit extracts at the initial and final stages were made and the values obtained are given in table II.

TABLE II

Changes in pH values during the experiments

pH	Oranges			Grapefruit			Lemons		
		Natural			Natural		Natural		
Initial	2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
Final	2.7	3.8	4.1	2.8	3.7	4.2	2.8	3.9	4.1

It is evident that the pH increased slightly in all cases except for the sample brought initially to pH 4.5. In the latter the pH decreased to 4.1.

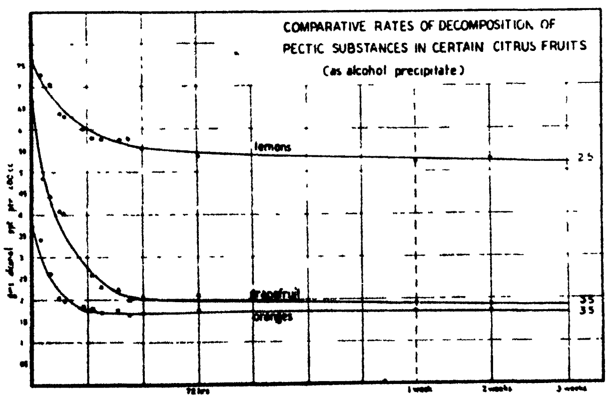


Fig. 3. Comparative rates of decomposition of pectic substances in certain citrus fruits. Expressed as alcohol precipitate.

The increase in pH is probably due to a change in the nature of the colloidal buffering substances present, while the decrease may be due largely to formation of free pectic acids by demethoxylation. The changes in pH, however, are not very large and may be neglected in view of the other changes involved. The results may be assumed to have been obtained at substantially constant pH value.

Comparison of citrus fruit extracts at their natural pH value

The rate of change in amount of alcohol precipitate obtained (by the A.O.A.C. procedure (**1**)), from samples of oranges, grapefruit, and lemons is shown in table III and in the corresponding graph (fig. 3). The data for

TABLE III

Rates of change in pectins of oranges, grapefruit and lemons determined as alcohol precipitate

Time of storage before determination	Grams alcohol precipitate per 200 ml. of filtrate		
	Oranges	Grapefruit	Lemons
hr.	*gm.*	*gm.*	*gm.*
0	0.375	0.691	0.775
4	0.343	0.488	0.728
8	0.263	0.442	0.705
12	0.205	0.433	0.640
14	0.199	0.408	0.633
22	0.185	0.403	0.598
26	0.179	0.262	0.581
30	0.175	0.229	0.577
38	0.176	0.226	0.572
42	0.169	0.200	0.576
48	0.170	0.209	0.552
72	0.173	0.214	0.539
168	0.174	0.190	0.527
336	0.166	0.186	0.532

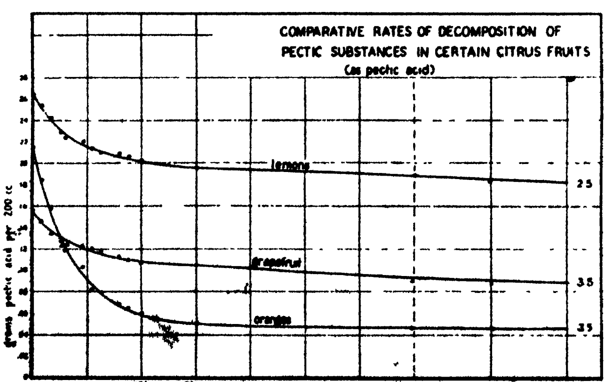


FIG. 4. Comparative rates of decomposition of pectic substances in certain citrus fruits (as pectic acid).

the pectin content determined as pectic acid are given in table IV and are represented by the graph shown in figure 4.

TABLE IV

RATES OF CHANGE IN PECTINS OF ORANGES, GRAPEFRUIT AND LEMONS DETERMINED AS PECTIC ACID

TIME OF STORAGE BEFORE DETERMINATION	GRAMS PECTIC ACID PER 200 ML. OF FILTRATE		
	ORANGES	GRAPEFRUIT	LEMONS
hr.	*gm.*	*gm.*	*gm.*
0	0.214	0.157	0.264
4	0.185	0.146	0.254
8	0.157	0.134	0.242
12	0.127	0.128	0.229
14	0.119	0.127	0.225
22	0.102	0.122	0.220
26	0.082	0.120	0.215
30	0.079	0.118	0.211
38	0.068	0.114	0.210
42	0.065	0.110	0.205
48	0.060	0.108	0.204
72	0.051	0.106	0.195
168	0.047	0.093	0.188
336	0.046	0.087	0.184

EFFECT OF pH ON THE RATE OF DECOMPOSITION OF CITRUS PECTINS

The changes in pectins observed in macerates adjusted to varying pH values are shown in table V and figures 5, 6, 7, 8, and 9.

TABLE V

CHANGES OF PECTIC SUBSTANCES IN FRUITS AT VARYING pH VALUES

TIME OF STORAGE BEFORE DETERMI-NATION	GRAMS PECTIC ACID PER 200 ML.								
	ORANGES			GRAPEFRUIT			LEMONS		
	pH:—2.5	3.5	4.5	2.5	3.5	4.5	2.5	3.5	4.5
hr.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0		0.214			0.157		0.264		
4	0.185	0.185	0.185	0.146	0.146	0.146	0.254	0.254	0.254
8	0.163	0.157	0.164	0.138	0.134	0.130	0.242	0.247	0.240
12	0.135	0.127	0.130	0.134	0.128	0.126	0.229	0.232	0.219
14	0.128	0.119	0.120	0.132	0.127	0.124	0.225	0.224	0.208
22	0.120	0.102	0.112	0.127	0.122	0.117	0.220	0.206	0.200
26	0.107	0.082	0.100	0.120	0.120	0.117	0.215	0.200	0.193
30	0.100	0.079	0.094	0.122	0.118	0.115	0.211	0.200	0.183
38	0.093	0.068	0.085	0.120	0.114	0.108	0.210	0.187	0.181
42	0.087	0.065	0.081	0.118	0.110	0.105	0.205	0.186	0.180
48	0.079	0.060	0.073	0.117	0.108	0.109	0.204	0.183	0.179
72	0.065	0.051	0.060	0.112	0.106	0.099	0.195	0.176	0.175
168	0.065	0.047	0.054	0.107	0.089	0.087	0.188	0.172	0.172
336	0.067	0.046	0.054	0.096	0.087	0.084	0.184	0.164	0.165
504	0.061		0.053	0.092		0.069		0.161	0.158

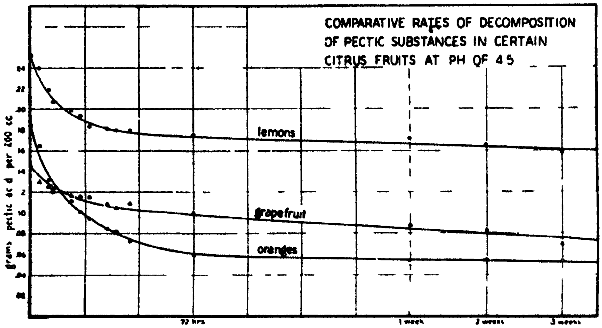


FIG. 5. Comparative rates of decomposition of pectic substances in certain citrus fruits at pH 4.5.

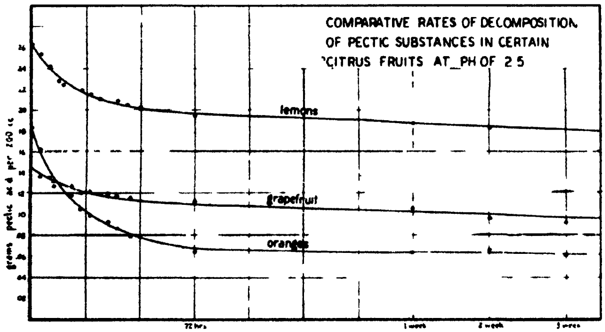


FIG. 6. Comparative rates of decomposition of pectic substances in certain citrus fruits at pH 2.5.

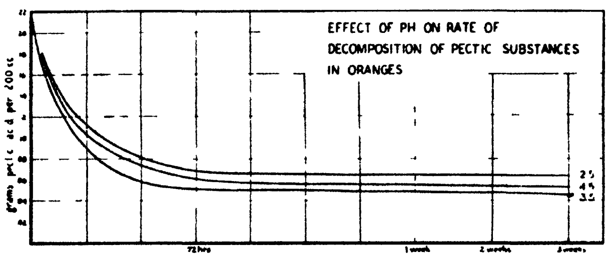


FIG. 7. Effect of pH on rate of decomposition of pectic substances in oranges.

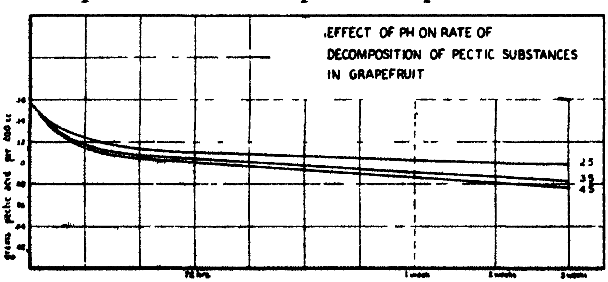


FIG. 8. Effect of pH on rate of decomposition of pectic substances in grapefruit.

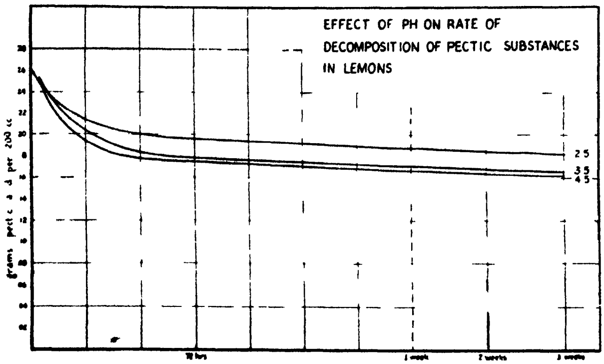


FIG. 9. Effect of pH on rate of decomposition of pectic substances in lemons.

Discussion

From the data shown in tables III and IV and the corresponding graphs (figs. 3 and 4) it is seen that the amount of soluble pectin in lemons is greater than that in oranges, and the amount in oranges exceeds that in grapefruit. A decrease in the amount of soluble pectin present in all three fruits occurs with increase in time of storage. It is of interest to note that this decrease is reflected in the extent of clarification of the filtrates from the different fruits as shown in figure 2.

On successive filtrates from each fruit at the natural pH value, there was marked clarification which was very rapid in oranges and more gradual in both grapefruit and lemons. In lemons the turbidity persisted throughout. The rate of decrease in the amount of unaltered pectic substance is shown by the graphs given in figures 3 and 4. It may be observed that a similar curve is obtained for each fruit; and that the rate of decrease in alcohol precipitate follows that of pectic acid. These graphs also show that for all fruits the initial rapid loss of soluble pectic substances occurred in approximately the same time.

In general, the decomposition of pectic substances measured as alcohol precipitate shows the same trend as that determined as pectic acid. If, however, we compare the ratio of alcohol precipitate to pectic acid precipitate in the three fruits after different time intervals, we observe a great variation as shown in table VI.

The ratio of alcohol precipitate to pectic acid remained approximately constant until after 22 hours, at which time most of the decomposable pectin is lost. In oranges the ratio then increases, indicating that although the gums and other non-pectinous water-soluble alcohol-precipitable materials were hydrolyzed at the same rate as pectin initially, they were decomposed at a slower rate thereafter. In lemons the ratio remained practically constant, thus indicating, either that the alcohol precipitable

TABLE VI

RATIO OF ALCOHOL PRECIPITATE TO PECTIC ACID IN SUCCESSIVE DETERMINATIONS

TIME OF STORAGE BEFORE DETERMINATION	RATIO OF ALCOHOL PRECIPITATE TO PECTIC ACID		
	ORANGES	GRAPEFRUIT	LEMONS
hr.			
0	1.8	4.4	2.9
4	1.85	3.3	2.8
22	1.8	3.2	2.7
48	2.8	1.9	2.7
72	3.4	2.0	2.7
168	3.7	2.6	2.8
336	3.6	2.7	2.8

material was all pectin or that the gums were decomposed at the same rate as pectin throughout. In the case of grapefruit the evidence indicates a more rapid decomposition of gums than of pectins in the initial stages followed by a less rapid decomposition.

It is seen that the total amount of soluble pectic substances decomposed varied greatly in the three citrus fruits. While in oranges almost all of this substance was decomposed, in grapefruit only about half of it had undergone such decomposition, and in lemons the amount decomposed was only one-third of the total. The reasons for this difference in behavior of the three fruits can only be surmised. Either there are definite differences in the nature and activity of the pectic enzymes present or there are differences in the pectins present. Although, as will be shown, the difference in pH had a measureable effect, it did not account for all the differences observed.

In figures 5 and 6 are shown the comparative rates of decomposition of pectic substances in the three fruits at pH values 2.5 and 4.5 respectively. In addition to the observation that in all cases the rate of decomposition was greater in oranges than in grapefruit or lemons, a comparison of the two graphs shows a greater decomposition of the pectic substances in all the fruits at the higher pH value.

With oranges, figure 7 indicates that the pectic substances were more rapidly decomposed at pH 3.5 than at either pH 2.5 or 4.5. The effect of the change of pH is more noticeable during the first three days; after this, the rates of decomposition approach constancy, a phenomenon represented by the parallelism of the curves.

With grapefruit (fig. 8), although the rate of decomposition was comparatively slow, it is seen that a pH of 4.5 was favorable for the decomposition. There is a marked difference in the rate of decomposition at pH 2.5 and 4.5, although the difference was smaller in the comparison between the rate of decomposition at pH 3.5 and 4.5.

The same results were obtained with lemons with the exception that the

rate of hydrolysis of pectic substance in lemons was faster than in grapefruit (fig. 9).

It is of interest to note that the effect on the decomposition of increasing the pH of each fruit from 2.5 to 4.5 is reflected in the degree of clarification in the extracts. On successive filtrates from each fruit at the upper pH range, the same progressive clarification was observed. This clarification is more apparent at the higher pH than at the normal or at the lower. In all cases, extracts obtained from fruit maintained at the lower pH value showed greater turbidity.

A study of pectin methoxylase activity in citrus fruits (to be presented elsewhere) indicated that this behaved similarly to that found in tomatoes by KERTESZ (**14**), *i.e.*, that its optimum pH was close to neutrality. In extracts from oranges or in pectin solutions to which these extracts were added, there occurred a very rapid decrease in viscosity but only a gradual increase in acidity owing to demethoxylation. This may indicate that a depolymerization of the pectin molecule precedes demethoxylation.[2]

Summary and conclusions

The rate of decomposition of natural pectins by the naturally occurring pectic enzymes was studied in various fruits. In apples the total pectin remained essentially constant for a period of about four days and then rapidly decreased to about two-thirds of its original value and remained at that level. In oranges the total pectin rapidly decreased to about one-third of its original value in the course of about 24 hours and then remained essentially constant. In cucumbers the decrease in pectin was fairly rapid and continuous.

A comparison of the rate of hydrolysis in oranges, grapefruit, and lemons indicated that:

1. The natural pectins hydrolyze more rapidly in orange than in grapefruit. In lemons the decomposition was slowest of the three.
2. In all cases, the rate of hydrolysis approached the maximum at the end of one day, decreased rapidly during the next two days and then gradually diminished.
3. The decomposition of pectin in the fruits was accompanied by the clarification of their extracts.

The effect of varying the pH of the extracts from oranges, grapefruit, and lemons was shown to be as follows:

1. The decomposition of pectic substance was found to be faster at pH

[2] Z. I. KERTESZ (Pectic enzymes IV. Structural considerations in connection with the enzymic hydrolysis of pectin. Jour. Am. Chem. Soc. 61: 2544–2548. 1939.) in a recently published paper reported also that "when pectin solutions are enzymatically decomposed the viscosity decreases more rapidly than corresponds to the increase in reducing power."

3.5 for oranges, and at 4.5 for both grapefruit and lemons, than at the other pH values tested.
2. The rate of decomposition at pH 2.5 for all fruits was much slower than at higher ranges.
3. The greatest effect of varying the pH value was observed in the early stages of the experiment.

Division of Fruit Products
University of California
Berkeley, California

LITERATURE CITED

1. Assoc. Offic. Agr. Chem. Official and tentative methods of analysis. Fourth edition. P. 324–325. 1935.
2. Beaven, G. H., Hirst, E. L., and Jones, J. K. N. Pectic substances. Part IV. Citrus araban. Jour. Chem. Soc. **1939**: 1865–1868.
3. ——————, and Jones, J. K. N. Molecular structure of pectic acid. Chemistry E Industry **58**: 363. 1939.
4. Bonner, James. The chemistry and physiology of the pectins. Bot. Rev. **10**: 475–497. 1936.
5. Branfoot, M. H. A critical and historical study of the pectic substances of plants. Food Investigation, Special Report no. **33**: 1–154. 1929. His Majesty's Stationery Office, London.
6. Ehrlich, Felix. Zur Kenntnis der Pektolase. Enzymologia **3**: 185–199. 1937.
7. Freudenberg, Karl. Polysaccharides and lignin. Ann. Rev. Biochem. **8**: 81–112. 1939.
8. Gaddum, L. W. The pectic constituents of citrus fruits. Florida Agr. Exp. Sta. Bull 268. 1934.
9. Harvey, E. M., and Rygg, G. L. Field and storage studies on changes in the composition of the rind of Marsh grapefruit in California. Jour. Agr. Res. **52**: 747–787. 1936.
10. Hirst, E. L., and Jones, J. K. N. Pectic substances. Part I. The araban and pectic acid of the peanut. Jour. Chem. Soc. **1938**: 496–505. 1938.
11. ——————, and ——————. Pectic substances. Part II. Isolation of an araban from the carbohydrate constituents of the peanut. Jour. Chem. Sec. **1939**: 452–453.
12. ——————, and ——————. Pectic substances. Part III. Composition of apple pectin and the molecular structure of the araban component of apple pectin. Jour. Chem. Soc. **1939**: 454–460.
13. Kertesz, Z. I. Pectic enzymes. Ergeb. Enzymforsch. **5**: 233–250. 1936.

14. ———. Pectic enzymes. II. Pectic enzymes of tomatoes. Food Res. **3**: 481–487. 1937.
15. LEVENE, P. A., MEYER, G. A., and KUNA, MARTIN. Preliminary note on the mode of union of the galacturonic acid residues in pectic acid. Science n.s. **89**: 370. 1939.
16. MYERS, PHILIP B., and BAKER, GEORGE L. Fruit jellies. VIII. The rôle of pectin. 4. The physico-chemical properties of pectin. Delaware Agr. Exp. Sta. Bull. 187. 1934.
17. NORMAN, A. G. Studies in pectin. III. The degree of esterification of pectin in the juice of the lemon. Biochem. Jour. **22**: 749–752. 1928.
18. ———. Biochemistry of pectin. Sci. Prog. **24**: 263–279. 1929.
19. ———. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford. 1937.
20. NORRIS, F. W. Pectic substances of plants. IV. The pectic substances in the juice of oranges. Biochem. Jour. **20**: 993–997. 1926.
21. ———. Review on pectin in Ann. reports on the progress of chem. **35**: 362–365. The Chemical Society, London. 1939.
22. RIPA, R. Die Pektinstoffe. Braunschweig. 1937.
23. ROTHSCHILD, HANS. Studies on pectins. IV. Isolation of pectic enzymes. Enzymologia **5**: 359–364. 1939.
24. RYGG, G. L., and HARVEY, E. M. Behavior of pectic substances and naringin in grapefruit in the field and in storage. Plant Physiol. **13**: 571–586. 1938.
25. SMITH, F. Molecular structure of pectic acid. Chemistry E Industry, **58**: 363–364. 1939.
26. STUEWER, R., BEACH, N. M., and OLSEN, A. G. Pectin studies. II. Sugar-acid-pectin relationships and their bearings upon routine evaluation of apple pectin. Ind. Eng. Chem., Anal. Ed. **6**: 143–146. 1934.
27. SVEDBERG, THE, and GRALEN, NILS. Carbohydrates of well defined molecular weight in plant juices. Nature **142**: 261–262. 1938.

MOVEMENT OF ORGANIC SOLUTES IN THE SAUSAGE TREE, *KIGELIA AFRICANA*[1]

HARRY F. CLEMENTS

(WITH ONE FIGURE)

Introduction

In recent years, considerable emphasis has been placed on the concept that organic materials, assumed to be sugar, move *en masse* with the water in which they are dissolved through phloem tissues to points of storage or utilization. The mass flow hypothesis, as this concept has been labelled, was suggested independently by MÜNCH (9) and CRAFTS (3) in recent times, although HARTIG (7) in 1858 and SACHS (10) in 1865, proposed ideas somewhat similar. No attempt has been made, however, to subject this concept to an objective examination. It is the purpose of this paper to study the growth and development of the fruits of the sausage tree in light of this concept and to determine whether or not the hypothesis can explain the movement as observed in this plant.

The mass flow concept

Before presenting the data collected, it is perhaps necessary to re-state the mass flow concept. MÜNCH (9) proposed that the sugar solution moves through the sieve tubes of the phloem from the point of source (storage organ or photosynthetic tissues) to the point of utilization. CRAFTS (3) broadens the concept of the tissues involved to the whole phloem including sieve tubes, companion cells and parenchyma, and further proposes that the sugar solution in its movement traverses not only the lumina of the cells but the cell walls as well. No data are presented demonstrating either of these postulates except observations associated with the exudation of liquid material obtained from the phloem area when this tissue is cut. It has been assumed that this exudation represents movement in the *intact* plant. COOIL (2) has shown, however, that the only significance which can be attached to this exudation phenomenon is that it represents the readjustment of tissue tensions following the disruption caused by cutting the phloem as well as the associated parenchyma and xylem tissues. The fact that most species do not show exudation at all has been claimed by CRAFTS (4) to be due to the inadequate technique of the investigator. He shows that if any species is cut and the wounded portion dipped into water, streaming can be observed from the exposed surface and he assumes that this is a demonstration of streaming in the uninjured plant. Such streaming, however, may

[1] Published by permission of the Director of the Hawaii Agricultural Experiment Station as technical paper no. 74.

mean one of two other things: (1) that two materials of different specific gravities and viscosities have been mixed—the heavier one sinks and because of its greater viscosity gives the impression of streaming; (2) that exudation from the cut sieve tubes is induced as a result of dipping the exposed tissue in water, thereby causing a localized osmotic movement resulting in the displacement of sieve tube cytoplasm by the absorbed water entering by way of the parenchymatous cells associated with the sieve tubes. At any rate, the sugar solution is presumed to move through phloem tissue very much as the transpiration stream moves through the xylem.

The second point on which no pertinent data have been presented involves the concentration of the solution which moves. Although he presents no data obtained by chemical analysis showing the amount of sugar actually present in the exudate, CRAFTS assumes that at least two-thirds of the dry material collected in the exudate is sugar, and therefore that the phloem sap is at least a 10 per cent. sugar solution. SACHS (**10**), however, in 1865 showed that the dry matter in the sieve tube of cucurbit was largely nitrogenous in nature and that only a very small amount of sugar appears in the tube. In fact, this led him to propose that sugars did not move in the sieve tubes at all and that only nitrogenous compounds move there, while sugars moved in the associated parenchyma tissues. COOIL (**2**) recently completely substantiated these compositional observations. About one-third of the dry material of the exudate is simple protein, and sugar constitutes about 0.5 per cent. of the fresh weight.

The third point involves the time in the ontogeny of the phloem element at which this movement takes place or at least when it is most active. CRAFTS has shown by means of plasmolysis and dye absorption that as the sieve tube ages, it becomes more and more permeable. Whether any significance can be attached to these observations is beside the point. The significant point is that this is taken to mean that the sieve tube is essentially passive in the movement of material. Therefore, it seems clear that the old sieve tubes should be more effective in transport than the young tubes which are thoroughly alive, and therefore far from passive.

The fourth point of the hypothesis is that once the sugar solution reaches the point of storage or utilization, the water passes into the xylem system and thus circulates back through the plant. No data are presented to support the assumption that this normally occurs in plants.

Although there still remains the matter involving the movement of water in large quantities from xylem and mesophyll into the phloem, this particular point, although again shown clearly in diagrams, has not been shown to occur in plant tissues in volume sufficient to account for movement throughout the phloem. This point, however, is not cogent to this paper, and hence will not be pressed here.

Thus, briefly, the mass flow hypothesis states that a concentrated sugar solution (at least 10 per cent.) moves *en masse* through passive phloem tissues and the water, after dropping its sugar, returns by way of the xylem tissues.

Experiment

The fruits of the sausage tree (*Kigelia africana*, Bentham) are good subjects for this type of research. On each inflorescence, about eight fruits form, but abscission claims half of these. Four fruits may remain on a single floral stem and develop to maturity. There may be more than a hundred such clusters on a tree. At maturity, the floral stem may be 150 cm. long, hanging from the tree (fig. 1) supporting four large fruits, each one

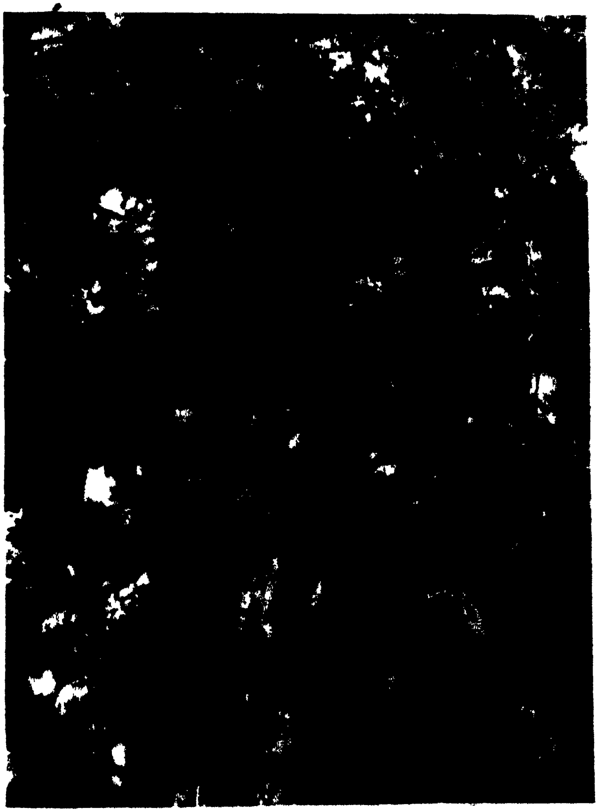


FIG. 1. The fruits of *Kigelia africana* showing one cluster of four fruits.

of which may weigh five pounds or more. To be sure, many of the clusters have fewer than four fruits, especially since the tree is very rare in the northern hemisphere and souvenir hunters are numerous.

On June 9, 1939, the flowers were hand pollinated and on June 18, 1939, several clusters were selected for observations. The floral stem of one of these clusters was girdled. All the fruits of this cluster stopped growth and soon dropped. The usual interpretation would be that by breaking the continuity of the phloem food was stopped and hence the fruits dropped.

Another cluster of fruits was enclosed in a bag made of black paper. The object here was to prevent photosynthesis from taking place since the fruits are normally green when young. That the treatment was effective was indicated by the fact that the enclosed fruits were quite without green color. Thus, all of the food which this fruit consumed in growth and storage had to move into it via the floral stem.

Three other clusters were tagged but were without treatment. Two of these were interfered with by tourists and were abandoned.

Beginning June 18, 1939, and continuing at intervals until September 10, each fruit of each cluster was measured by determining its length and circumference. Since the fruits are solid and essentially round in cross-section, it is easy to get a fairly accurate measure of the volume. At the time each group of measurements was made, a fruit corresponding in size to those being measured was selected from the tree, measured, weighed, dried and again weighed. In this way, it was possible to determine the weight of the experimental cluster after each interval and also to calculate therefrom the increase in dry matter for each period.

Phloem samples from the floral stem were gathered at three intervals. The phloem area is very clearly marked and it was easy to remove the xylem tissues as well as the tissues external to phloem. Microscopic examination revealed that the tissue remaining was phloem only. These samples were dried and preserved for sugar analysis.

Results

Only the results obtained from the bagged cluster of fruits will be presented here. Actually, bagging made no difference in the development of the fruit. The unbagged cluster showed no significant difference over the bagged fruit. The record of the volume growth of the four fruits which were bagged are reported in table I.

Each time measurements were made, fruits comparable to those being measured were removed, and their volume, green wight, and dry weight obtained. From these data, the green weights and dry weights of the bagged fruits were calculated. These data are shown in table II.

Although no one has ever demonstrated the form in which organic carbon moves, it has generally been assumed to be sugar. Very probably some of the carbon enters a fruit as amino acids, but the total nitrogen content of these fruits is 1.41 per cent. of the dry weight. If the carbon asso-

TABLE I

VOLUME RECORD FOR ONE CLUSTER OF FOUR FRUITS

DATE OF MEASUREMENT	VOLUME TOTAL	INCREASE OVER PREVIOUS MEASUREMENT	INCREASE IN VOLUME PER DAY
	ml.	*ml.*	*ml.*
June 18, 1939	619	619	68*
June 25	2128	1509	215
July 2	4573	2444	349
July 9	6346	1772	253
July 15	7634	1287	214
July 21	8450	816	136
July 29	9355	905	113
August 9	9716	361	32
August 20	9871	154	14
August 29	9948	77	8
September 10	9979	30	2

* Calculated from the date of pollination, June 9, 1939.

ciated with this nitrogen moved as amino acids rather than sugar, the total amount of carbon thus moved would still be considerably less than 5.0 per cent. of the total carbon required for the fruit. Even though the possibility of translocatory compounds other than sugar exists, in this work the assumption that sugars are the important carbon carriers into these fruits will be maintained.

In order, therefore, to obtain a sugar value for the dry material which

TABLE II

GREEN AND DRY WEIGHTS FOR ONE CLUSTER OF FOUR FRUITS

DATE	GREEN WEIGHT PER ML.	TOTAL GREEN WEIGHT OF FRUITS	DRY MATTER	TOTAL DRY WEIGHT	INCREASE IN DRY WEIGHT	DAILY INCREASE IN DRY WEIGHT
	gm.	*gm.*	%	*gm.*	*gm.*	*gm.*
June 18, 1939	1.117	692	11.9	82.3	82.3	9.1
June 25	1.037	2207	11.9	262.8	180.5	25.8
July 2	0.933	4267	11.5	490.7	227.9	32.6
July 9	0.957	6073	11.3	686.3	195.6	27.9
July 15	0.957	7305	11.3	825.6	139.3	23.2
July 21	0.957	8086	11.3	913.8	88.2	14.7
July 29	0.913	8541	12.1	1033.5	119.7	14.9
August 9	0.890	8648	14.1	1219.3	185.8	16.9
August 20	0.883	8716	15.4	1342.3	123.0	11.2
August 29	0.887	8824	17.4	1535.5	193.2	21.5
September 10	0.879	8771	18.7*	1640.3	104.8	8.7

* On November 15, 1939, a fruit was taken from the tree. The dry weight had increased only 0.5 per cent. indicating that by September 10, growth was very nearly completed.

moved into the fruit, a total carbon analysis of the fruit was made.[2] This was found to represent 43.46 per cent. of the dry material. In table III, the total amount of carbon moving into the fruit each day is reported, and, granting that carbon moves into a fruit in the form of sugar, it is simple to convert the daily additions of carbon to the fruit into a hexose sugar equivalent. These values are also shown in table III.

TABLE III

DAILY GAIN IN DRY WEIGHT EXPRESSED AS CARBON AND AS HEXOSE SUGAR

Date	Daily gain in dry weight expressed as	
	Carbon	Hexose sugar equivalent
	gm.	*gm.*
June 18, 1939	3.95	9.87
June 25	11.21	28.03
July 2	14.17	35.42
July 9	12.12	30.30
July 15	10.08	25.20
July 21	6.39	15.98
July 29	6.48	16.20
August 9	7.34	18.35
August 20	4.87	12.17
August 29	9.34	23.35
September 10	3.80	9.50

Thus, the figures given in the "hexose sugar" column represent the amount of sugar which each day moves into the fruit by way of the long floral stem. To this should be added the amount of sugar which is respired each day. Respiration measurements were not made during these studies, but a value could be arrived at from some similar types of fruits. On the basis of such calculations between two and three grams of sugar are respired each day. But since such values represent about 10 per cent. of the sugar moving into these fruits, the sugars used in the respiratory activity are ignored in this study. It is reasonable to assume, however, that respiration per unit volume is much higher in the young fruits than in the old fruits.

In summarizing this phase of the study, it is clear that by far the greatest increase in fruit volume is accomplished during the first five weeks, for it is during this period that the largest daily increments are observed. During this same period the daily increments of dry material are higher than those observed after the floral stem is more mature. During this early period, the tissues of the floral stem are young; in fact, the long stem (145 cm.), which for the most part is really a single internode, is still elongating. One would expect the metabolic rate of this tissue to be considerably higher than that

[2] The author is indebted to Mrs. RUTH YOSHIDA of the Soils Division for this determination.

during the later two-month period. Hence, it appears that the greatest deposition of carbohydrate material in the fruit occurs during the time when the phloem elements of the floral stem are young. This relationship is probably less coincidental than it is causal.

The next phase of this study involves a determination of the strength of the sugar solution which obtains in the phloem of the floral stem at the time of fruit development and, since the total amount of sugar necessary to provide the dry material actually observed moving into the fruits each day is known, it becomes a simple matter to determine the total volume of solution which would be required to move the sugar into the fruit were the mass flow hypothesis the correct interpretation of the mechanism involved.

On July 9 and again on July 15, during the peak of the fruit growth, and finally on September 10, phloem samples were taken from the floral stem by removing xylem tissues as well as the outer paranchymatous tissues. This separation was made easy by a ring of fiber groups just outside the phloem region. Microscopic examination showed the sample to be made up of phloem only. Stained-preparations showed a large number of sieve tubes and companion cells, some sclerenchyma and a large amount of parenchyma. Microchemical tests for reducing sugars as well as sucrose revealed moderate concentrations in the inner phloem and somewhat lighter concentrations in the older phloem.

The tissue was dried, and analyzed for reducing sugars and total sugars. The results of this study are reported in table IV.

In the last column of table IV, the amount of sugar in 100 gm. of fresh material is calculated in terms of percentage solutions. Since it is assumed

TABLE IV

SUGAR CONTENT OF THE PHLOEM TISSUE*

DATE OF COLLECTION	DRY WEIGHT	REDUCING SUGARS AS PERCENTAGE DRY WT.	AMOUNT OF SUGAR PER 100 GM. FRESH WT.	PERCENTAGE SOLUTION
		REDUCING SUGARS		
	%	%	gm.	%
July 9, 1939	11.3	5.28	0.597	0.672
July 15	11.6	4.35	0.505	0.571
September 10	24.4	1.73	0.422	0.558
		TOTAL SUGARS		
July 9, 1939	11.3	14.00	1.582	1.78
July 15	11.6	13.11	1.521	1.71
September 10	24.4	11.38	2.777	3.67

* The author is indebted to Mr. BRUCE COOIL for these determinations.

by CRAFTS that the whole phloem area is involved in transport then presumably all the water in the phloem is mobile. Hence, the sugar present must be expressed on a solution basis. Reducing sugars which probably are the most important in transport are present in low concentrations. The reducing sugar values obtained here average 0.62 per cent. and correspond closely to the values obtained by COOIL (2) for the exudate of cucurbits but are vastly different from the 10 per cent. assumed by CRAFTS.

Now, it is hardly likely that the parenchyma of the phloem is even fractionally as active in translocation as the sieve tube, if at all. According to COOIL, the petiole tissues of a carbohydrate-high plant are found to be very high in sugar while in a nitrogen-high plant, the tissue sugars are low. Yet, the amount of sugar in the exudate of the phloem is about the same. This probably means that the sugar in the parenchyma is storage material. Sucrose is probably more of a storage sugar than a translocatory sugar. Despite these considerations, however, to give the benefit of all doubts to a favorable interpretation of the mass flow hypothesis, the amount of water required to move the sugar into the fruit during each day is calculated on the basis of the reducing sugar as well as the total sugar concentrations. The sugar values for the collections July 9 and 15 serve as the basis for these calculations, since these represent the period of maximum deposition in the fruit. Unfortunately, a collection of phloem tissue was not made on July 2.

TABLE V

AMOUNT OF SOLUTION NECESSARY TO TRANSPORT THE REQUISITE AMOUNT OF SUGAR INTO THE FRUITS PER DAY

DATE	GRAMS OF SOLUTION	
	0.62 PER CENT. SOLUTION	1.75 PER CENT. SOLUTION
	gm.	*gm.*
June 18, 1939	1592	564
June 25	4521	1602
July 2	5713	2024
July 9	4887	1731
July 15	4064	1440
July 21	2577	913
July 29	2613	926
August 9	2960	1049
August 20	1963	695
August 29	3766	1334
September 10	1532	543

It is, therefore, apparent (table V) that, during the time of maximum fruit development, enormous quantities of solution (5.7 liters of a 0.62 per cent. solution or 2.0 liters of a 1.75 per cent. solution) would have to be forced through the phloem each day. The cross-sectional area of the phloem

tissue was only 0.53 $cm.^2$, and the floral stem was 145 cm. long, not including the 15 cm. length of stem which connected the main floral branch to each individual fruit. In table VI, the amount of water required to move the necessary amount of sugar into the fruits is put on the basis of 1 $cm.^2$ of phloem cross-sectional area.

TABLE VI

RATE OF SOLUTION MOVEMENT REQUIRED PER SQ. CM. OF PHLOEM AREA

DATE	RATE OF SOLUTION MOVEMENT			
	AS 0.62 PER CENT. SOLUTION		AS 1.75 PER CENT. SOLUTION	
	PER DAY	PER HOUR	PER DAY	PER HOUR
	gm.	*gm.*	*gm.*	*gm.*
June 18, 1939	3009	125	1066	44
June 25	8545	356	3028	126
July 2	10797	449	3825	159
July 9	9236	384	3272	136
July 15	7681	320	2722	113
July 21	4871	203	1726	71
July 29	4939	205	1750	72
August 9	5594	233	1983	82
August 20	3710	154	1314	54
August 29	7117	296	2521	105
September 10	2895	120	1026	42

Thus, depending upon the strength of the sugar solution, the rate of solution movement at the peak of fruit development would have to be 159.4 or 449.1 gm. of solution per $cm.^2$ of phloem *per hour.* Now DIXON (5) in his studies of water transport in xylem tissues finds that a movement of 1.93 grams of H_2O per $cm.^2$ of xylem per hour was sufficient to account for the speeds of water transport in *Taxus.* FARMER (6) reports that the xylem of certain angiospermous trees delivers 20 ml. of water per hour per unit of cross-sectional area. It is hardly reasonable to believe that water movement in the phloem is many times faster than in xylem systems.

One further point remains to be shown. In table V is shown the daily amount of water necessary to move the requisite amounts of sugar into the fruit. It is necessary to follow this water and determine its ultimate disposition. Each day, according to the mass flow hypothesis, a certain volume of water moves through the floral stem into the fruits. Here the sugar is removed from the solution, and the water is then eliminated from the fruit to make room for more. According to the hypothesis, this water returns by way of xylem tissue. Some of the water, however, would remain to make up the daily volume increase and of course some is lost in transpiration. The amount lost in transpiration is small, since the fruits hang in the shade of the tree. According to measurements, transpiration accounts for less than 20

gm. per day per fruit and, in view of the large amount of water involved, can be ignored. The increases in volume are taken into account in table VII.

TABLE VII

AMOUNTS OF WATER RETURNING FROM FRUIT

DATE	AMOUNTS OF H_2O MOVED INTO FRUIT EACH DAY,—CALCULATED AS		VOLUME INCREASE EACH DAY	AMOUNT OF H_2O WHICH MUST RETURN VIA XYLEM	
	A 0.62 SOL.	A 1.75 SOL.			
	gm.	*gm.*	*ml.*	*Column 1 minus 3*	*Column 2 minus 3*
June 18, 1939	1592	564	69	1523	495
June 25	4521	1602	216	4305	1386
July 2	5713	2024	349	5364	1675
July 9	4887	1731	253	4634	1478
July 15	4064	1440	215	3849	1225
July 21	2577	913	136	2441	777
July 29	2613	926	113	2500	813
August 9	2960	1049	33	2927	1016
August 20	1963	695	14	1949	681
August 29	3766	1334	9	3757	1325
September 10	1532	543	3	1529	540

Thus, if the solution moves into the fruit as a 0.62 per cent. solution, 5.4 liters of water would have to return via xylem tissues each day (week ending July 2). If the solution were a 1.75 per cent. solution then 1.7 liters would have to return. Even the smaller figure involves an hourly average of 69.8 ml. The larger value would involve a backward movement of 223.5 ml. per hour. To test this point, a cluster of fruits was selected on July 9. A longitudinal cut was made through the bark of the floral stem, the xylem was carefully loosened, then cut, and the fruit end of the cut xylem was led out through the incision.

Thus, with the phloem intact, the water returning through the xylem should be intercepted at this point, and with such amounts as 69.8 ml. or 223.5 ml. per hour, the water should literally spout out especially since the radius of the xylem is about 1 mm. But after waiting three hours during late morning, with no sign of water coming out of the cut xylem, this project was discontinued.

At this point it is well to compare the findings of this paper with the assumptions underlying the mass flow hypothesis. The assumption that sugars move in a solution involves the movement of such tremendous quantities of water through poorly adapted tissues that it is inconceivable that any protoplasm no matter how permeable or passive could remain in the sieve tubes and other cells of the phloem. The necessary rates of movement are many times greater than have been observed in xylem tissues.

The assumption that the protoplasm of the conducting tissues is passive is not based on fact. In these studies there is a much greater movement of sugar during the early stages of floral stem development than there is later on. At this stage the tissues are young and certainly more active metabolically than later on. If the movement of sugar were correlated with passivity of protoplasm, it should occur late, and yet such is not the case.

The assumption that sugar in the phloem tissue exists as a 10 per cent. solution is a gross exaggeration; for the exudate of cucurbits as well as the phloem tissue of the sausage tree, the value is less than one per cent.

The assumption that the water which carries the sugar into the fruit returns by way of the xylem could not be verified in these studies.

Thus, it would appear that the mass flow hypothesis is hopelessly inadequate in accounting for the growth and development of the fruits of *Kigelia africana*.

On the other hand, it is much simpler to conceive of the sugar moving in solution but independently of the more or less static solvent. That this movement is associated with the activity of protoplasm is indicated by the fact that the greater part of the growth and development of the fruit takes place while the sieve tubes are young and at a higher metabolic level than later on. The amounts of sugar moving are so great that it seems that the living protoplasm expends energy in aiding the translocation of sugars (**1**, **8**).

Summary

1. The growth of the fruits of *Kigelia africana* was followed from the day of pollination until maturity to determine the required rate of sugar movement into them.

2. An attempt was then made to explain this movement on the assumptions of the mass flow hypothesis.

3. The hypothesis was found to be inadequate in the following particulars:

   a. Although a passive sieve tube cytoplasm is necessary to the hypothesis, the greatest movement of materials into the fruit was observed during the first five weeks of development.

   b. Although the strength of the moving sugar solution has been assumed to be at least a 10 per cent. solution, in these studies the phloem tissue has a reducing sugar level of 0.62 per cent. and a total sugar level of 1.75 per cent.

   c. No evidence could be obtained relating to the return of water from the fruit through xylem tissue even though enough time was allowed for the theoretical return of as much as 209 to 670 ml.

   d. On the basis of the sugar content of the phloem tissue, to account for the observed movement of sugar into the fruit, such enormous quan-

tities of solution (10,797 ml. of a 0.62 per cent. or 3825 ml. of a 1.75 per cent. per day per cm.[2] of phloem cross-sectional area) would have to move that it is inconceivable that any cytoplasmic organization would remain within the phloem tissue. In fact, the necessary speed of solution delivery into the fruit would be many times the capacity of xylem tissues.

4. The observed movement of sugar into the fruit is so great that it seems necessary to describe it as a function of the living protoplasm of the sieve tube which through its respiratory activity does work in the movement.

University of Hawaii
Honolulu, T. H.

LITERATURE CITED

1. Clements, Harry F. Translocation of solutes in plants. Northwest Science **8**: 9–21. 1934.
2. Cooil, B. The significance of phloem exudate of *Cucurbita pepo* with reference to translocation of organic materials. (In press.)
3. Crafts, A. S. Movement of organic materials in plants. Plant Physiol. **6**: 1–42. 1931.
4. ————. The relation between structure and function of the phloem. Amer. Jour. Bot. **26**: 172–177. 1939.
5. Dixon, H. H. Transpiration and the ascent of sap. Macmillan. 1914.
6. Farmer, J. B. On the quantitative differences in the water conductivity of the wood in trees and shrubs. Proc. Roy. Soc. London **B90**: 218–250. 1918.
7. Hartig, Th. Ueber den Herbstsaft der Holzpflanzen. Bot. Zeit. **16**: 369–370. 1858.
8. Mason, T. G., and Maskell, E. J. Oxygen supply and the activation of diffusion. Ann. Bot. **50**: 455–499. 1936.
9. Münch, E. Versuche über den Saftkreislauf. Ber. d. bot. Ges. **45**: 340–356. 1927.
10. Sachs, Julius. Lectures on the physiology of plants (Trans. by H. M. Ward). pp. 353–365. 1887.

DETERMINATION OF AMMONIA, GLUTAMINE, AND ASPARAGINE AMIDE NITROGEN IN PLANT JUICE[1]

FRANK S. SCHLENKER

(WITH ONE FIGURE)

The most generally used method for the determination of preformed ammonia in plant material is the aspiration of a sample with $Ca(OH)_2$, MgO, or 52 per cent. K_2CO_3. This technique was shown to include other than preformed ammonia nitrogen (4) for, with prolonged aeration, there was a continual evolution of ammonia, owing to the presence of unstable nitrogen compounds. PUCHER (3) reported that this was caused by the presence of amides.

This error was obviated in plant juice by the removal of ammonia by permutit, washing free from excess juice, treating with NaOH, and aspirating in the Van Slyke-Cullen ammonia apparatus.

CHIBNALL and WESTALL (1) and VICKERY, *et al.* (5), have shown that it is possible to determine glutamine and asparagine amide nitrogen in a mixture of the two amides by choosing the proper conditions for hydrolysis. In an attempt to collect a number of methods into a system for the analysis of plant juice it was thought worth-while to study the adsorption of amides by permutit, and to reinvestigate the use of permutit for the determination of ammonia.

Determination of ammonia

The action of 52 per cent. K_2CO_3 on plant juice under continued aeration causes a breakdown of the amides present. A typical curve for this, using beet juice, is shown in figure 1 (curve D), the break coming at the end of two hours.

An attempt was made to simulate this curve with a synthetic solution of the following composition: 0.0812 mg. NH_3–N, 1.5 mg. asparagine amide–N, 0.959 mg. glutamine amide–N and 1 ml. buffer[2] per 5-ml. sample. In figure 1, each curve, excepting curve D, represents the effect of aerating this mixture or variations of it, lacking glutamine or asparagine or both, with 10 ml. of 52 per cent. K_2CO_3 for varying periods. A study of these

[1] Published by permission of the Director as Contribution No. 563 of the Rhode Island Agricultural Experiment Station.

[2] Modified buffer (2)

7 parts 0.1 N KH_2PO_4 / 1 part 0.1 N K_2HPO_4		2.74 ml.
M/3 sodium malate		2.17 ml.
Glucose		1.00 gr.
Water	dilute to	15.0 ml.

curves indicates, as might be expected, that the break occurs when ammonia nitrogen has been completely removed, that is, after approximately two hours (curve A). From curve B, it is evident that the hydrolysis of asparagine is slight, hence the large amount of ammonia given off as shown in curve C must come from glutamine. The rate of hydrolysis of the amides at the time of complete ammonia nitrogen removal can be obtained from a consideration of the curves at the two-hour period.

Curve A	Ammonia	0.0812 mg. Ammonia–N
Curve B	Ammonia and asparagine	0.0829 mg. Ammonia–N
Curve C	Ammonia, asparagine, and glutamine	0.1012 mg. Ammonia–N

Using these figures it can be calculated from curve C that the total amount of ammonia-nitrogen evolved at the end of two hours is due to 0.0812 mg. NH_3–N, 0.0183 mg. glutamine amide nitrogen, and 0.0017 mg. asparagine amide nitrogen. The rate of glutamine hydrolysis for this time interval is approximately ten times that of asparagine.

Permutit method for ammonia determination

Sodium permutit is the usual reagent used for the estimation of am-

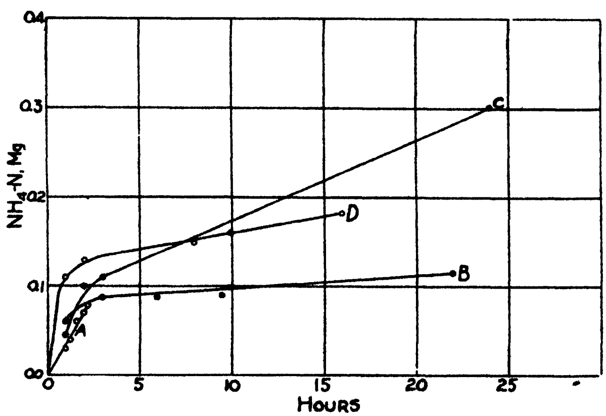


Fig. 1. Curve A, 0.0812 mg. NH_3–N.
Curve B, 0.0812 mg. NH_3–N + 1.5 mg. asparagine amide nitrogen.
Curve C, 0.0812 mg. NH_3–N + 1.5 mg. asparagine amide nitrogen + 0.959 mg. glutamine amide nitrogen.
Curve D, beet leaf juice.

monia. Various other permutits were prepared and studied (table I).

These results fall in line with the lyotropic series, sodium being the ion most easily substituted, excepting lithium which was not studied. Here hydrogen permutit is somewhat out of place as it is usually the most difficult ion to displace. Sodium permutit appears to be the desirable form to use.

TABLE I

RECOVERY OF AMMONIA FROM WATER SOLUTION BY DIFFERENT PERMUTITS

PERMUTIT	PERCENTAGE RECOVERY OF 0.0812 MG. AMMONIA–N
Na	99.0
Ca	92.0
H	90.0
Mg	83.7

In view of WHITEHORN's conclusions (6) that permutit adsorbs amines and amino acids, a number of tests was made to determine the adsorption of glutamine and asparagine by permutit. A synthetic glutamine solution, several portions of which were adjusted to different pH values, containing 1 ml. of the previously described buffer solution and 0.959 mg. of glutamine amide nitrogen per 3 ml. were shaken with 2.5 ml. permutit for 2.5 min., the permutit was then washed and aspirated in presence of NaOH (table II).

TABLE II

NITROGEN ADSORBED FROM GLUTAMINE SOLUTIONS CONTAINING 0.959 MG. GLUTAMINE AMIDE N BY PERMUTIT CALCULATED AS PERCENTAGE OF AMIDE NITROGEN

	SOLUTION						
	1	2	3	4	5	6	7
pH of the solution ...	6.31	6.00	5.81	5.50	5.04	4.62	3.15
Percentage nitrogen adsorbed...	1.1	0.8	1.5	0.8	1.3	1.1	1.5

Similar mixtures were prepared, using asparagine, and adsorption studies were made under conditions like those for glutamine (table III).

TABLE III

AMMONIA NITROGEN ADSORBED FROM ASPARAGINE SOLUTIONS BY PERMUTIT, CALCULATED AS PERCENTAGE OF AMIDE NITROGEN

ASPARAGINE AMIDE NITROGEN	pH 8.25	pH 7.17	pH 6.50	pH 6.44	pH 5.91	pH 5.49	pH 5.42	pH 5.15	pH 4.60	pH 3.82
mg.	%	%	%	%	%	%	%	%	%	%
0.0382	....	....	....	0.79	0.79	1.31	....	....	0.79	0.00
0.0955	0.00	0.31	0.00	....	....	....	0.52	1.04	....	....
0.1910	4.20	0.63	0.37	1.57	2.67	2.36	2.10	2.25	2.50	2.50
0.3820	....	....	....	0.00	1.31	1.25	....	....	2.15	2.20
0.5730	0.98	0.98	0.26	....	....	....	1.06	1.06	....	....
0.9050	0.39	0.38	0.34	....	....	....	0.77	1.17	....	....
1.9100	....	....	....	0.38	0.60	0.58	....	....	0.53	0.58

The results of a single experiment at one concentration of glutamine gives evidence of glutamine adsorption which appears, with the exception of two pH values, to be constant over the pH range used. A more detailed study of the adsorption of asparagine indicates that the least amount of adsorption takes place at pH 6.50.

The results with pure solutions show that, in the presence of these two amides, the permutit method for ammonia should give slightly high results. For example, synthetic solutions, containing varying amounts of buffer, ammonia, and asparagine were shaken with 2.5 or 0.5 ml. of permutit for 2.5 min., washed free of excess sample, and then aspirated. In most cases recoveries of ammonia somewhat higher than theoretical were obtained. Although no attempt was made to recover ammonia in the presence of glutamine it is quite obvious that, in view of the results presented in table II, such experiments would give results slightly higher than theoretical.

The low values obtained in the fifth and sixth columns, table IV, may

TABLE IV

RECOVERY OF AMMONIA IN PRESENCE OF ASPARAGINE

BUFFER*	ASPARAGINE	PERMUTIT	PERCENTAGE NH_3–N RECOVERED		
			0.0406 MG. NH_3–N ADDED	0.0812 MG. NH_3–N ADDED	0.2030 MG. NH_3–N ADDED
ml.	*mg.*	*ml.*	%	%	%
1	0.382	2.5	101	103	103
1	0.382	0.5	103	101	106
2	0.764	2.5	104	103	103
2	0.764	0.5	103	98	96
3	1.146	2.5	104	100	95
3	1.146	0.5	103	92	94

* See footnote 2, p. 701.

be explained by the fact that amounts of permutit used, 2.5 and 0.5 ml., represent a total of only 2.5[3] and 0.51 milliequivalents of exchange surface, respectively. It seems likely that with an increasing salt concentration, but with a constant amount of exchange surface, the adsorption of ammonia decreases under these conditions.

Determination of glutamine and asparagine

To determine glutamine, a juice sample obtained from leaf tissue frozen with CO_2, is hydrolyzed for two hours at pH 6.00 to 6.50, while for total amides a three-hour period in 1 N H_2SO_4 is used. Both reactions are carried out in a boiling water bath. Because of the high acid medium of the total

[3] The permutit used in this work has a total exchange capacity of 230 m.e. per 100 grams and 2.5 ml. of it weighs approximately one gram.

amide determination, it is necessary that proteins be removed from juice samples, otherwise there is a liberation of ammonia from the juice proteins during the period of hydrolysis. The conditions for glutamine hydrolysis are not sufficiently severe to cause juice protein to hydrolyze, at least with the material studied here. Table V shows both amides as determined in

TABLE V

EFFECT OF FREEZING WITH SOLID CO_2 ON GLUTAMINE AND ASPARAGINE DETERMINATIONS

FREEZING TIME	TOMATO LEAF JUICE	
	GLUTAMINE AMIDE–N	ASPARAGINE AMIDE–N
Min.	*mg./ml.*	*mg./ml.*
0	0.025	0.063
10	0.025	0.033
20	0.024	0.021
30	0.024	0.023
90	0.025	0.023
120	0.026	0.020
150	0.025	0.021

juice expressed directly from tomato leaves. The decrease in the asparagine nitrogen figure is perhaps to be attributed to the precipitation, at an extremely low temperature, of a hydrolyzable protein fraction.

Three methods of precipitating the juice protein fraction were tried: namely, heating at 70° C., Folin's sodium tungstate reagents, and 10 per cent. tannic acid. The values in table VI indicate that any one of the

TABLE VI

ASPARAGINE AND GLUTAMINE AMIDE NITROGEN IN PROTEIN-FREE JUICE*

SAMPLE	HEATED TO 70°		10 PER CENT. TANNIC ACID‡		SODIUM TUNGSTATE§	
	GLUTAMINE	ASPARAGINE	GLUTAMINE	ASPARAGINE	GLUTAMINE	ASPARAGINE
	mg./ml.	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./ml.*
1	0.0344	0.0080	0.0375	0.0268†	0.0325	0.0096
2	0.0367	0.0045	0.0399	0.0065	0.0340	0.0066

* Preformed ammonia was removed by shaking with permutit in all determinations.
† Incomplete precipitation of proteins.
‡ 10 per cent tannic acid, 5 ml. per 10 ml. juice.
§ Sodium tungstate 10 per cent., 1 + 1 with 0.662 N H_2SO_4, 5 ml. per 10 ml. juice.

methods, when properly used, gives results comparable to the other two; however, heat precipitation of protein is by far the most convenient. Tannic acid is satisfactory except that it contains ammonia which is imparted to the solution when the reagent is used in excess. Unless this added ammonia is removed the results for amides are higher than their true values.

The determination of ammonia in juice clarified by the three methods shown in table VI (each reagent added in excess) compared to untreated juice is as follows: 96.1 per cent., 113.6 per cent., and 98.8 per cent., respectively, showing a slight adsorbing effect of heat-coagulated protein and tungstate precipitate, as well as the influence of excess tannic acid on the ammonia content of juice after treatment. Owing to this loss of ammonia by adsorption on heat-coagulated protein it is necessary to remove the total amount of preformed ammonia nitrogen prior to the amide determination. In some instances the heat treatment is insufficient to remove all protein, for it has been observed that in the total amide determination a coagulum sometimes appears, for example with spinach juice. This can be avoided by adding one drop of 85 per cent. H_3PO_4 to the juice before heating it to 70° C.

Experiments made by adding glutamine and asparagine to tomato leaf juice show adequate recovery of these amide forms of nitrogen (table VII).

TABLE VII

RECOVERY OF GLUTAMINE AND ASPARAGINE AMIDE NITROGEN ADDED TO TOMATO LEAF JUICE

Glutamine		Asparagine	
Added	Recovered	Added	Recovered
mg./ml.	*mg./ml.*	*mg./ml.*	*mg./ml.*
0.0415	0.0400	0.0350	0.0380
0.0831	0.0760	0.0700	0.0720
0.1995	0.2010	0.1400	0.1290

Recommended methods of analysis

The plant sample is cut into small sections, placed in a cheesecloth bag, and frozen by a direct stream of solid CO_2 as it is formed at the end of a coil of tubing attached to a tank of liquid CO_2. After the excess CO_2 has evaporated from the frozen tissue the sample is pressed, and the juice obtained.

Ammonia

To 2.5 ml. of sodium permutit in an ammonia aspiration tube, add an aliquot of the juice sample, (1 to 5 ml.) shake for 2.5 min. and then wash free from excess juice, thus eliminating asparagine and glutamine. The tubes are placed in the Van Slyke-Cullen aspiration block and 3 to 4 ml. water, and two drops of caprylic alcohol (an antifoam reagent) and 3 ml. 10 per cent. NaOH are added. The apparatus is connected in the usual manner and aspirated for two hours. The ammonia is caught in 0.01 N acid and determined either by Nesslerization or titration, preferably by Nesslerization.

GLUTAMINE AND ASPARAGINE

Place 10 ml. of juice in a centrifuge tube, add one drop of 85 per cent. H_3PO_4 (if necessary) heat to 70° C., cool, and centrifuge. Shake a portion of clear supernatant liquid with 2.5 ml. of permutit to remove preformed ammonia. Pipette 1 ml. of the ammonia-free juice into an ammonia tube containing 10 ml. of a phosphate buffer[4] at pH 6.40 and hydrolyze in a boiling water bath for two hours. Cool, place in aspiration block, add 2 to 3 drops of caprylic alcohol and 2 ml. of 52 per cent. K_2CO_3, aspirate two hours, and determine ammonia from glutamine by Nesslerization. Although aspiration with K_2CO_3 causes the hydrolysis of a small quantity of asparagine, the error involved is probably not much greater at this point than it would be if permutit were used to remove the hydrolyzed glutamine ammonia from the buffer mixture containing unhydrolyzed asparagine. Thus, to simplify the procedure, no attempt is made to remove the ammonia coming from glutamine by the use of permutit. One ml. of the ammonia-free sample is placed in another ammonia aspiration tube containing 1 ml. 6 N H_2SO_4 and 4 ml. water. This is hydrolyzed three hours in a boiling water bath, and cooled. Treat with sufficient 10 per cent. NaOH to make alkaline to phenolphthalein and total amide nitrogen is determined. Total amide nitrogen minus glutamine nitrogen gives asparagine amide nitrogen, on the assumption that these are the only amides present.

Discussion

The results in tables II and III showing the adsorption of small amounts of ammonia from glutamine and asparagine solutions by permutit, may be explained by the adsorption of the entire amide molecule with subsequent hydrolysis of the amide nitrogen under the alkaline conditions of the ammonia determination during aspiration, or by the adsorption of ammonia coming from the hydrolysis of the amides in solution. Experiments to test the first possibility, involving the determination of amino nitrogen in an asparagine solution before and after treatment with permutit were inconclusive because of the small amounts of amino nitrogen present.

WHITEHORN (6) has shown that such amides as urea and its derivatives, certain amino acids, and glutamic acid hydrochloride are not adsorbed by permutit; in fact, his data indicate that only relatively strong bases are removed from solution.

Thus by accepting the indirect evidence that amide molecules are not adsorbed by permutit, the second supposition proposed above points towards the actual conditions, that is, the ammonia adsorbed by permutit when shaken with an asparagine or glutamic solution comes from the hydrolysis of the acid amide group and there is evidence to show that these amides do hydrolyze in solution.

[4] 50 ml. M/5 KH_2PO_4, 12.60 ml. M/5 NaOH, dilute to 200 ml.

If a freshly prepared glutamine solution is tested for the presence of ammonia no color appears when the Nessler reagent is added. One standing, however, even at room temperature, hydrolysis takes place and finally a strong Nessler reaction becomes evident. An asparagine solution will react in a similar manner, but more slowly, for even after two days the color reaction, though positive, is faint.

The high results for the ammonia determinations as shown in table IV, which can be considered as an error, and likewise would be expected to produce low results in the determination of the amides, may be no error at all. This can be illustrated as follows: In a plant system consisting of amides (A) and preformed ammonia (B) the conditions might be—

$$A - R\text{-}CONH_2 + 2H_2O \rightleftarrows R\text{-}COOH + NH_4OH \rightleftarrows NH_4^+ + OH^-$$
$$B - \qquad NH_4OH \rightleftarrows NH_4^+ + OH^-$$

Thus, the ammonia derived from reaction A is adsorbed by permutit (as is the free ammonia from other sources) and it can be considered accordingly, as preformed ammonia. In this system the amide nitrogen, determined by the methods presented here, would be less than that represented by the unhydrolyzed amide of A. This, however, would not necessarily be in error for, as is pointed out, this ammonia-nitrogen is considered as preformed ammonia, not as amide nitrogen. This is, of course, based on the assumption that the time of shaking plant juice with permutit, 2.5 min., is not long enough to shift the amide nitrogen-ammonia nitrogen equilibrium to the right to any great extent.

Conclusions

1. Sodium permutit under proper conditions is capable of removing quantitatively the preformed ammonia of plant juice.
2. Glutamine amide nitrogen can be determined in the presence of asparagine amide nitrogen by the use of proper conditions for hydrolysis.
3. Asparagine amide nitrogen can be estimated as the difference between total amide nitrogen and glutamine amide nitrogen.
4. Methods are proposed for these determinations.

The writer wishes to express his appreciation to Dr. H. B. Vickery for the glutamine used in this study.

Rhode Island Agricultural Experiment Station,
Kingston, Rhode Island

LITERATURE CITED

1. Chibnall, A. C., and Westall, R. G. The estimation of glutamine in the presence of asparagine. Biochem. Jour. 26: 122–132. 1932.
2. Hurd-Karrer, A. Titration curves of etiolated and of green wheat seed-

lings reproduced with buffer mixtures. Plant Physiol. **5**: 307–328. 1930.

3. Pucher, G. W. Reported at meeting of New England Section of American Society of Plant Physiologists, Amherst, Mass. May 25–26, 1934.
4. Schlenker, F. S. Comparison of existing methods for the determination of ammonia nitrogen and their adaptability to plant juices. Plant Physiol. **7**: 685–695. 1932.
5. Vickery, H. B., Pucher, G. W., Clark, H. E., Chibnall, A. C., and Westall, R. G. The determination of glutamine in the presence of asparagine. Biochem. Jour. **29**: 2710–2720. 1935.
6. Whitehorn, J. "Permutit" as a reagent for amines. Jour. Biol. Chem. **56**: 751–764. 1923.

SOME CHEMICAL AND RESPIRATIONAL CHANGES IN THE PAPAYA FRUIT DURING RIPENING, AND THE EFFECTS OF COLD STORAGE ON THESE CHANGES[1]

WINSTON W. JONES AND HISASHI KUBOTA

(WITH THREE FIGURES)

Papaya (*Carica papaya*) is a plant long established in Hawaii, having been brought to the Islands before 1823. It has been under study by the Hawaii Agricultural Experiment Station since 1902, but most of the investigations have been on cultural requirements and selection. The rapid rise in commercial importance of the fruit in recent years makes it necessary to know something of the changes occurring in the fruit during ripening, as a basis for harvesting, storage, and shipment.

Review of literature

THOMPSON (**13**) gives some of the early analyses of the papaya and shows that in the ripe fruit there is very little sucrose but a high percentage of reducing sugars. Her study was of several different strains, namely: Trinidad, South African, Honolulu, Barbados, Panama, and Tahiti. These strains have since lost their identity, however, and have been known at the station by their accession numbers. POPE (**6**), in a study of changes occurring during ripening, found very little sucrose in any case but a marked increase in reducing sugars during ripening. He does not state the strain with which he worked. MILLER (**4**) gives the composition and some of the uses of the papaya. WARDLAW (**14**) has shown that at the beginning of ripening (*i.e.*, the beginning of the development of the yellow color) there is a marked increase in the production of carbon dioxide, which reaches a high point (climacteric peak) at the time of full ripeness. After this peak, there is a decrease in carbon dioxide evolution. He further shows that the carbon dioxide content of the fruit cavity increases with maturity. WILCOX (**15**) found that the papaya can be held in cold storage at 32° to 34° F. (0 to 1.5° C.) for 45 days, but the fruit is subject to decay. JONES (**1**) has shown that papayas can be held at 32° to 34° F. for at least 12 days if the fruit is ripe when placed in storage. The ripening process is apparently permanently stopped in unripe fruit held at this temperature.

Materials and methods

The fruits used in this investigation were all of the type IV (**10, 11**) Solo strain, and were grown in the Station's experimental plots at Kailua, Oahu.

[1] Published with the approval of the Director, Hawaii Agricultural Experiment Station as technical paper no. 57.

The fruits were harvested as needed. Owing to the nature of growth of the papaya, it is difficult to obtain a representative composite sample. In a few cases, a sample of three fruits was used but in most cases means of analyses of similar individual fruits were used.

Methods of analysis were standard: Sugars were determined by the method of STILES, PETERSON, and FRED (9); nitrogen, by the reduced iron method of PUCHER, LEAVENWORTH, and VICKERY (8), adapted to the Micro-Kjeldahl method described by PREGL (7); and carbon dioxide by the method of THOMAS (12) and MITCHELL (5).

Presentation of data

NORMAL RIPENING PROCESS

From the time of the beginning of ripening in the fruit until full ripeness is attained, the process is continuous. The first noticeable change is the development of yellow color in the funiculus. The color then spreads outward, and full ripeness is attained when the outer surface is completely yellow. Accompanying the change in color, the physical characteristics of the flesh change from very tough and rubbery to very soft and crushable.

The data presented in table I show the differences in concentration of sugars and other constituents of papaya harvested at three stages of maturity.

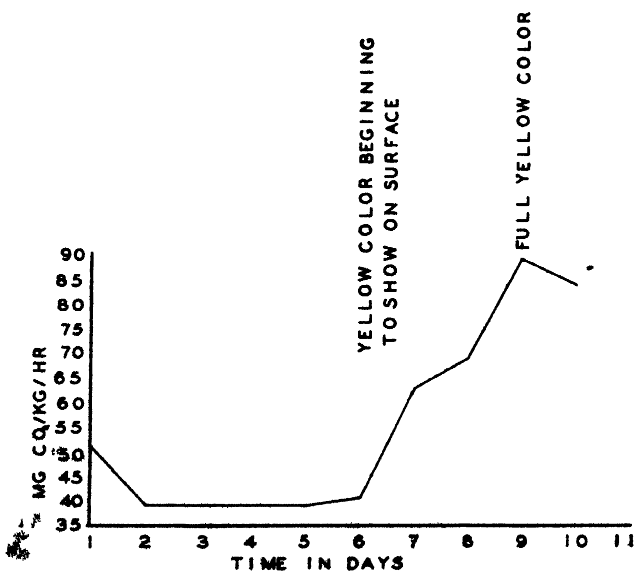


FIG. 1. Respiration of papaya fruit during ripening at approximately 25° C.

Figure 1 shows the carbon dioxide given off by fruit harvested mature-green and allowed to ripen at room temperature. Figure 2 shows the percentage of carbon dioxide and oxygen contained in the cavities of fruits harvested at different stages of maturity.

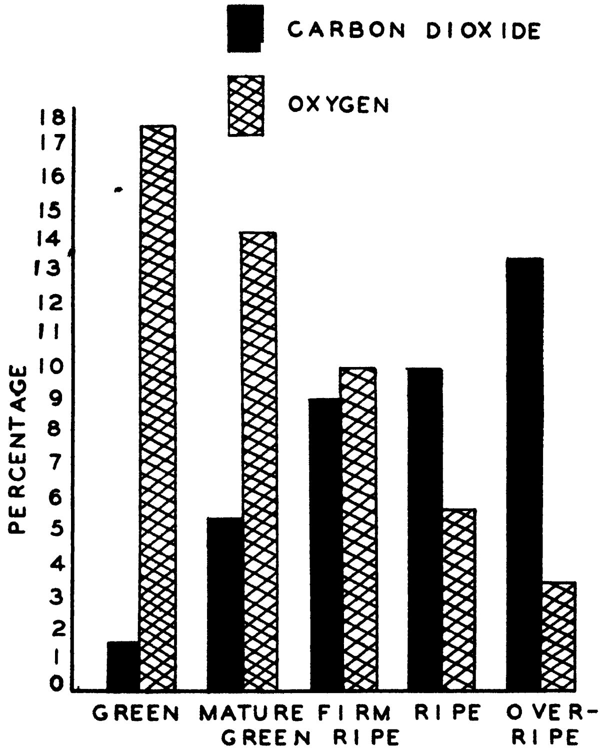


FIG. 2. Carbon dioxide and oxygen content of the gas in the internal cavity of the papaya at different stages of maturity.

CHEMICAL CHANGES OCCURRING DURING RIPENING

From table I it may be seen that during the ripening process a change occurs in the relationship between sucrose and reducing sugars. In the green fruit, 42 per cent. of the total sugar occurs as sucrose, while in the ripe fruit only 18 per cent. of the total sugar occurs as sucrose. The acid-hydrolyzable material drops from 9.33 per cent. of the total dry weight in the green fruit to 4.36 per cent. in the ripe fruit. As there is no starch present in the fruit, this decrease represents a change in cell-wall constituents, and accounts, at least in part, for the change in consistency of the flesh during ripening. There is apparently no significant change in the nitrogen content during ripening.

TABLE I

COMPOSITION* OF PAPAYA FRUIT AT DIFFERENT STAGES OF MATURITY

MATURITY	H_2O	SOLUBLE SOLIDS	REDUCING SUGAR	SUCROSE	TOTAL SUGAR	SOLUBLE NITROGEN	INSOLUBLE NITROGEN	TOTAL NITROGEN	ACID-HY-DROLYZABLE MATERIAL
	%	%	%	%	%	%	%	%	%
Ripe	85.3	11.56	62.64	14.40	79.04	0.28	0.25	0.53	4.36
Firm ripe	86.1	10.67	62.23	12.41	74.64	0.35	0.31	0.66	4.74
Mature-green	86.1	10.49	40.70	30.98	71.68	0.29	0.30	0.59	9.33

* Water and soluble solids as percentage of fresh weight; others as percentage of dry weight.

RESPIRATIONAL CHANGES OCCURRING DURING RIPENING

Carbon dioxide elimination and the carbon dioxide and oxygen content of the internal cavity were followed from the beginning of ripening to overripeness. The carbon dioxide elimination was measured on fruit harvested mature but green and allowed to ripen, while the internal gases were measured in fruit harvested at progressive stages of ripeness. Figure 1 shows the carbon dioxide elimination of approximately one dozen individual fruits measured separately. It will be noted that in the firm-ripe fruit (*i.e.*, with the yellow color just beginning to show) the rate of carbon dioxide elimination is represented by a low curve parallel with the base line. As the color develops, the rate of respiration, as measured by carbon dioxide elimination, rapidly increases and reaches a peak simultaneously with full color development. After this peak, the rate of respiration decreases. In some instances it increases again but this can be attributed to the growth of fungi on the overripe fruit and to the leakage of internal gases through the wounds caused by the fungi. Figure 2 shows that long before the development of external color and before the rate of external elimination of carbon dioxide begins to increase, the concentration of carbon dioxide in the internal atmosphere has begun. This increase in carbon dioxide in the internal atmosphere continues throughout the ripening period and past full ripeness. Thus, there is no peak of internal carbon dioxide concentration. As the carbon dioxide content of the internal cavity increases, the oxygen content decreases.

EFFECT OF COLD STORAGE ON THE OBSERVED RIPENING PROCESS

Fruit at three stages of ripeness—mature but green; firm ripe with yellow color just beginning to show; and ripe, with full yellow color but firm—were stored at 3° to 4° C. for 11 days. There were no indications of ripening during the period, and at the end of the cold storage treatment

there were no noticeable changes in the fruit. On removal to room temperature (approximately 25° C.) the green fruit did not ripen, but small sunken spots developed and in the course of 2 or 3 days the fruits were overrun with fungi. The firm-ripe fruit reacted very much as the green, never ripening normally. The ripe fruits were in essentially the same condition after cold storage as before but were subject to rapid breakdown and fungal attack when held at room temperature.

EFFECT OF COLD STORAGE ON THE CHEMICAL CHANGES OCCURRING DURING RIPENING

Table II shows the composition of mature-green fruit before and after cold storage, and when allowed to ripen at room temperature without any

TABLE II

COMPOSITION* OF PAPAYA FRUITS WHEN HARVESTED MATURE-GREEN, WHEN HARVESTED MATURE-GREEN AND ALLOWED TO RIPEN AT ROOM TEMPERATURE, AND WHEN HARVESTED MATURE-GREEN AND HELD FOR 11 DAYS AT 3°–4° C.

MATURITY	H_2O	SOLUBLE SOLIDS	REDUCING SUGAR	SUCROSE	TOTAL SUGAR	SOLUBLE NITROGEN	INSOLUBLE NITROGEN	TOTAL NITROGEN
	%	%	%	%	%	%	%	%
Mature-green	86.1	10.49	40.70	30.98	71.68	0.29	0.30	0.59
Harvested mature-green; ripe after 5 days at room temperature	86.9	9.96	56.22	8.00	64.22	0.44	0.34	0.78
Mature-green —11 days at 3°–4° C.	86.9	9.40	29.24	33.02	62.26	0.35	0.39	0.74

* Water and soluble solids as percentage of fresh weight; others as percentage of dry weight.

cold storage. It will be noted that, during 11 days at 3° to 4° C., there was no such hydrolysis of sucrose as occurred when fruit was allowed to ripen at room temperature. The quantitative differences in sugar content among the individual fruits are probably due to variability and should not be considered when comparing the treatments. The important point seems to be the relation between reducing sugars and sucrose.

EFFECT OF COLD STORAGE ON RESPIRATIONAL CHANGES

Figure 3 shows the carbon dioxide elimination of firm-ripe fruit stored at 3° to 4° C. during a period of 11 days. It will be noted that the rate of elimination changes very little over the 11-day period. It will also be noted that there is no climacteric rise in respiration during cold storage.

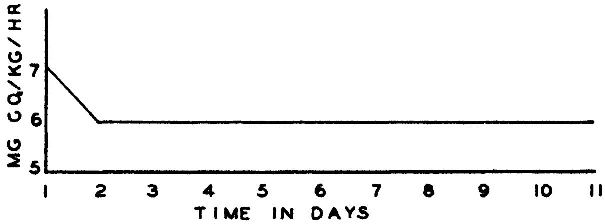


FIG. 3. Respiration of papaya fruits at 3° to 4° C.

Discussion

Ripening of the papaya, once begun, proceeds rapidly at room temperature. Within 3 or 4 days after the appearance of the first yellow color, the fruit is fully ripe. During this short period, several important changes occur: A large percentage of the sucrose is hydrolyzed, a climacteric rise in carbon dioxide elimination occurs, the flesh of the fruit changes from a very tough and rubbery to a soft, easily crushed texture, and the color changes from green to yellow. Unlike some other fruits, such as apples, the climacteric rise occurs in the papaya simultaneously with ripening and not before ripening.

Since the modification of the quarantine law to allow the entrance of Hawaiian fruits into the mainland after a cold treatment, the effects of cold storage on the papaya become of especial interest. The results presented show that cold storage halts the ripening processes in the papaya permanently rather than just for the duration of storage, as is the case with pears (**2**, **3**). On removal of the papaya from cold storage (11 days at 1.7° C.) to room temperature, small sunken dead spots develop in the course of 1 or 2 days. These dead spots serve as places of entrance for fungi; within 3 to 4 days the fruit is overrun with the fungi, and breakdown proceeds without ripening. These results agree with those reported by WARDLAW (**14**).

Summary

1. The normal process of ripening in the papaya is described and the effect of cold storage on this process discussed.

2. During the ripening process a large portion of the sucrose is hydrolyzed, the color changes from green to yellow, the flesh softens, and there is a rise in carbon dioxide elimination.

3. Cold storage stops the ripening process and causes a chill effect so that on removal to room temperature the ripening process is not resumed.

HAWAII AGRICULTURAL EXPERIMENT STATION
HONOLULU, T.H.

LITERATURE CITED

1. JONES, WINSTON W. Papaya storage. Hawaii Agr. Exp. Sta. Ann. Rept. 1937, pp. 28–30. 1937.

2. MAGNESS, J. R. Investigations in the ripening and storage of Bartlett pears. Jour. Agr. Res. **19**: 473–500. 1920.
3. MARTIN, WILLIAM E. Chemical study of the ripening process of Bosc pears. Bot. Gaz. **99**: 42–68. 1937.
4. MILLER, CAREY D., BAZORE, K., and ROBBINS, R. C. Some fruits of Hawaii—their composition, nutritive value and use. Hawaii Agr. Exp. Sta. Bull. 77. 1936.
5. MITCHELL, JOHN W. A method of measuring respiration and carbon fixation under controlled environmental conditions. Bot. Gaz. **97**: 376–387. 1935.
6. POPE, W. T. Papaya culture in Hawaii. Hawaii Agr. Exp. Sta. Bull. 61. 1930.
7. PREGL, FRITZ. Quantitative organic microanalysis. P. Blakiston's Son and Co., Inc., Phila.
8. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. & Eng. Chem. Anal. Ed. 2: 191–193. 1930.
9. STILES, H. R., PETERSON, W. H., and FRED, E. B. A rapid method for the determination of sugar in bacterial cultures. Jour. Bact. **12**: 427–439. 1926.
10. STOREY, W. B. The primary flower types of Papaya and the fruit types that develop from them. Proc. Amer. Soc. Hort. Sci. **35**: 80–82. 1938.
11. ————. Segregations of sex types in Solo Papaya and their application to the selection of seed. Proc. Amer. Soc. Hort. Sci. **35**: 83–85. 1938.
12. THOMAS, M. D. Precise automatic apparatus for continuous determination of carbon dioxide in air. Ind. & Eng. Chem. Anal. Ed. **5**: 193–198. 1933.
13. THOMPSON, A. The composition of Hawaiian fruits and nuts. Hawaii Agr. Exp. Sta. Rept. 1914: 62–75. 1915.
14. WARDLAW, C. W., and LEONARD, E. R. The storage and physiology of tropical fruits. Trop. Agr. **12**: 313–319. 1935.
15. WILCOX, E. V., and HUNN, C. J. Cold storage for tropical fruits. Hawaii Agr. Exp. Sta. Press Bull. 47. 1914.

A SEMI-MICRO METHOD FOR THE DETERMINATION OF REDUCING SUGARS

SAM G. WILDMAN AND ELMER HANSEN

(WITH THREE FIGURES)

Introduction

The MUNSON-WALKER method (3) with the modification of BERTRAND (1) has long been used for the determination of reducing sugars in plant extracts. The large amounts of solutions required by this method, however, make necessary the frequent preparation and standardization of solutions, which is inconvenient when large numbers of determinations are being made over an extended period of time. For plant materials containing not more than 10 to 12 per cent. sugar, it seems unnecessary to use the amounts of Fehling's solutions originally specified. ILJIN (2) in adapting the MUNSON-WALKER method to a micro scale, used 3-ml. portions of Fehling's solutions A and B. Where more than one per cent. is encountered, however, the dilutions made necessary by this modification in order to keep within his range of 1 to 25 mg. of sugar, result in too great a dilution factor in the final value. An additional advantage of reducing the amounts of reagents used would be in making possible carrying out the reduction and subsequent washing of the copper oxide precipitate directly in centrifuge tubes, thus eliminating the tedious and inconvenient procedure of filtering the precipitate on a Gooch crucible. The use of the centrifuge for micro-sugar analysis has been reported by ILJIN (2) and PHILLIPS (4).

Another objection to the MUNSON-WALKER method is the practice of boiling the sugar solutions over an open flame. QUISUMBING and THOMAS (5) have shown that this practice is subject to error, since at 100° C. the end-point of the copper reduction is taken on a continuously ascending curve, so that small changes in time or temperature will result in appreciable error. They pointed out the desirability of using a temperature at which the reduction curve would eventually become level, at which point the end-point could be taken. A temperature of 80° C. was found best for this purpose. SHAFFER and SOMOGYI (8) have also stressed the importance of taking the end-point of reduction on the level portion of the curve.

The use of standard potassium permanganate solution for titrating the reduced cuprous oxide according to the BERTRAND modification, has the disadvantages of instability of solution, sensitiveness to foreign organic matter and poor end-point, especially when the solutions cannot be thoroughly cleared. In view of the recent development of an inside indicator for potassium dichromate (7), it would appear desirable to use this solution instead of potassium permanganate for titration of the reduced copper oxide.

With the above considerations in mind, the MUNSON-WALKER-BERTRAND method has been modified to a semi-micro scale by incorporating the following changes:

(1) Using 20 ml. of Fehling's solution instead of 50 ml.
(2) Carrying out the sugar-copper reactions in an 80° C. water bath instead of boiling over an open flame.
(3) Centrifuging the Cu_2O precipitate instead of filtering on a Gooch crucible.
(4) Using potassium dichromate with sodium diphenylamine sulphonate inside indicator as a standard oxidant instead of potassium permanganate.

Preliminary experimentation

PREPARATION OF REAGENTS.—Fehling's solutions A and B were prepared according to the modification proposed by QUISUMBING and THOMAS (5) as follows:

Solution A was prepared by dissolving 69.28 gm. of c.p. $CuSO_4 \cdot 5H_2O$ in warm water and then diluting to 1 liter in a volumetric flask. After standing for several hours, the solution was filtered and transferred to a Pyrex reagent bottle.

Solution B was prepared by dissolving 346 gm. of c.p. sodium potassium tartrate in warm water and then transferring to a 1-liter volumetric flask. A saturated solution of sodium hydroxide was then digested on a steam bath until insoluble carbonates had settled out. The exact alkalinity of the solution was then determined and the amount containing exactly 130 gm. of sodium hydroxide added to the sodium potassium tartrate solution and made to 1 liter.

Ferric ammonium sulphate solution was made by dissolving 240.9 gm. of $Fe(NH_4)(SO_4)_2 \cdot 12\, H_2O$ in warm water, then adding 200 ml. of concentrated H_2SO_4. After cooling, the solution was diluted to 1 liter and filtered.

A 0.1000-N solution of potassium dichromate was prepared by dissolving 4.9037 gm. of highest purity $K_2Cr_2O_7$ (previously dried) in distilled water and then diluting to 1 liter in a volumetric flask.

Sodium diphenylamine sulphonate indicator was made by dissolving 0.32 gm. of barium diphenylamine sulphonate in 100 ml. of water, then adding 1.0 gm. of sodium sulphate and allowing the precipitate of barium sulphate to digest for one hour. The solution of sodium diphenylamine sulphonate was then filtered through a heavy filter paper.

DETERMINATION OF SUGAR-COPPER REDUCTION TIME.—QUISUMBING and THOMAS (5) have shown that 100 ml. of sugar-copper solutions held in an 80° C. water bath required 30 min. for complete reduction. Since smaller quantities of solutions were to be used, the possibility of reducing the time for reduction established by these investigators was investigated. Three

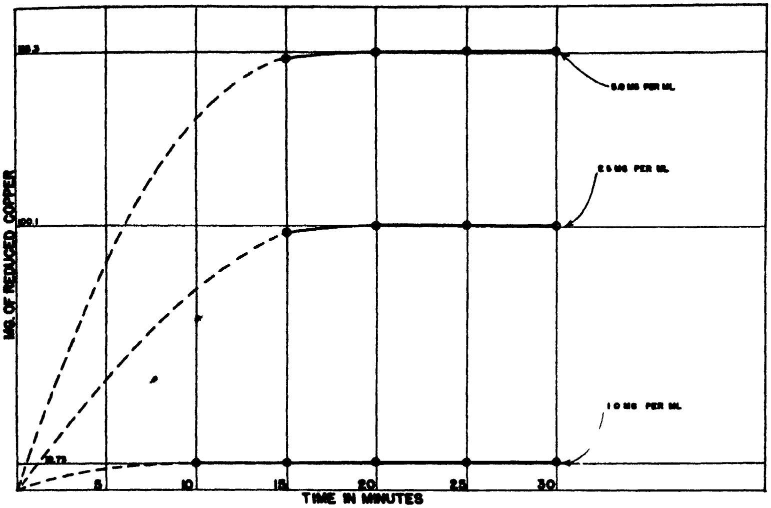


FIG. 1. Time required for digestion of varying concentrations of glucose and an alkaline copper tartrate solution in an 80° C. water bath.

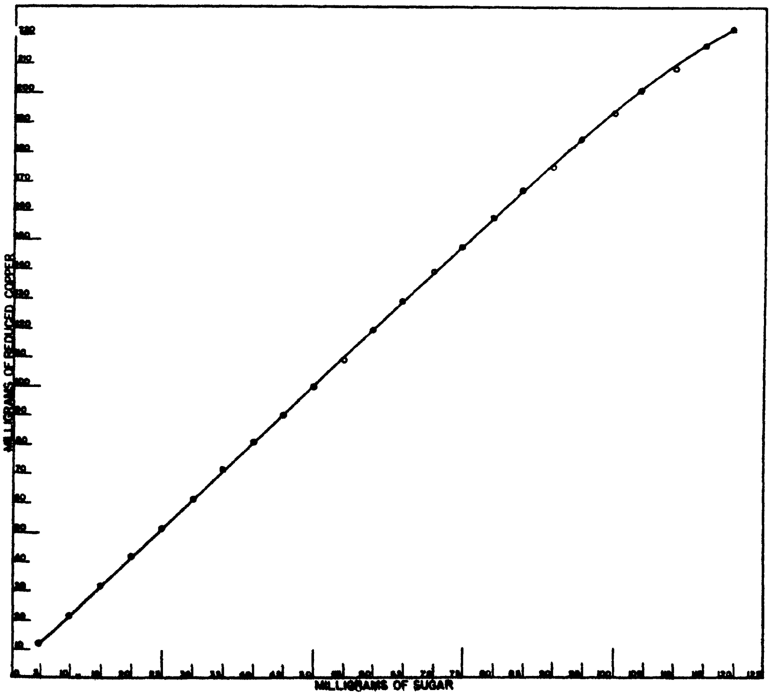


FIG. 2. Copper-glucose curve, determined by experiment.

glucose solutions were prepared containing 1 mg., 2.5 mg., and 5 mg. respectively per ml., representing the maximum, minimum, and median range of the sugar curve. After mixing with 20 ml. of Fehling's solutions, duplicate samples of the three sets were subjected to time intervals of 10, 15, 20, 25 and 30 minutes in an 80° C. water bath regulated to 1° C. by two bunsen burners. In this case, the results show (fig. 1) that the reduction curves became level shortly after 15 min., remained so at 20 to 25 min., and only slightly ascended in 30 min. In order to preserve uniformity, the time of 20 min. was arbitrarily accepted for further experimentation.

Arrangement of the centrifuge.—For these investigations an International type 1C centrifuge with an 8-place head carrying 50 ml. Pyrex tubes was used. To facilitate the rapid balancing of the centrifuge, the metal carriers were weighed and balanced to within 0.05 gm. by placing small pieces of sheet copper beneath the rubber cushions. The weight of the heaviest glass centrifuge tube was then determined, and the deficiencies in weight of the other seven, varying from 0.20 to 2.40 gm., recorded on the

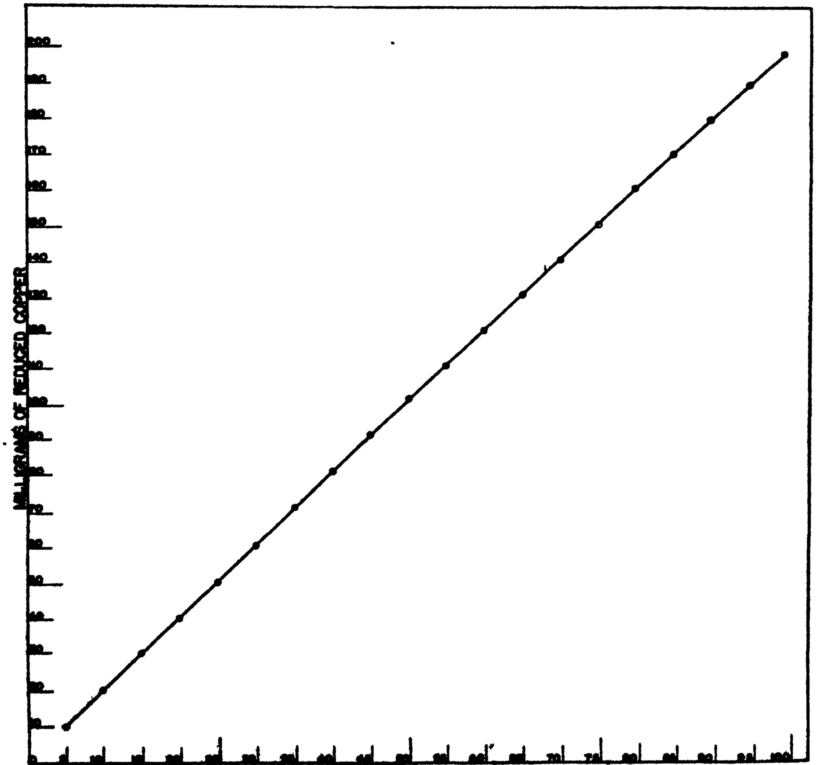


Fig. 3. Copper-sucrose curve, determined by experiment.

tubes with a glass-marking pencil. Hence, the centrifuge was rapidly balanced by the simple expedient of adding the same amount of water (measured from a burette set up for the purpose) in milliliters as recorded in grams on the centrifuge tubes. Since the centrifuge was designed to accommodate eight tubes at one time, two sets of eight tubes were prepared.

Method of analysis

As a result of preliminary experimentation, the following analytical procedure was adopted. To 20 ml. of sugar solution contained in a 50-ml. Pyrex centrifuge tube, measure from a burette 10 ml. each of Fehling's solutions A and B. Place in a water bath held at 80° C. (± 1° C.) for 20 min. While the first set of eight tubes is digesting, another set may be prepared to be placed in the water bath as soon as the first set is removed for centrifuging, etc. If this procedure is followed, 16 determinations may be made

TABLE I

GLUCOSE* $y = 0.4650x + 0.00028x^2$

COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$	COPPER	SUGAR	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*ml.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.33	0.78	85.0	41.55	13.31	165.0	84.37	25.85
10.0	4.67	1.57	90.0	44.12	14.10	170.0	87.04	26.68
15.0	7.04	2.38	95 0	46.71	14.90	175.0	89.94	27.25
20.0	9.41	3.14	100.0	49.30	15.70	180.0	92.78	28.21
25.0	11.80	3.93	105.0	51.92	16.49	185.0	95.65	29.00
30.0	14.20	4.71	110.0	54.55	17.25	190.0	98.48	29.80
35.0	16.62	5.50	115.0	57.18	18.05	193.3†	100.0	30.40
40.0	19.06	6.28	120.0	59.84	18.84	199.6	105.0	31.42
45.0	21.49	7.07	125.0	62.51	19.60	208.5	110.0	32.80
50.0	23.96	7.85	130.0	65.18	20.38	216.2	115.0	34.00
55.0	26.43	8.62	135.0	67.87	21.19	222.5	120.0	35.0
60.0	28.91	9.40	140.0	70.59	21.98			
65.0	31.41	10.20	145.0	73.33	22.75			
70.0	33.93	10.80	150.0	76.06	23.50			
75.0	36.46	11.74	155.0	78.80	24.25			
80.0	38.99	12.51	160.0	81.57	25.08			

* Sugar values for both glucose and sucrose were calculated from experimentally determined data (see fig. 2 and fig. 3) by the method of least squares according to the equations:

(1) $y = a + bx + cx^2$

(2) $b = \dfrac{\Sigma(x^4)\Sigma(xy) - \Sigma(x^3)\Sigma(x^2y)}{\Sigma(x^2)\Sigma(x^4) - [\Sigma(x^3)]^2}$

(3) $c = \dfrac{\Sigma(x^2)\Sigma(x^2y) - \Sigma(x^3)\Sigma(xy)}{\Sigma(x^2)\Sigma(x^4) - [\Sigma(x^3)]^2}$

The mean eror, calculated from the equation $d = \sqrt{\dfrac{\Sigma d^2}{n}}$ is 0.251 mg. for glucose and 0.228 mg. for sucrose.

† Values determined experimentally.

in approximately one hour. Remove the tubes and cool for 2 to 3 minutes in cold water. Equalize the weight of the tubes by adding water to each in accordance with its deficit in weight, and centrifuge at not less than 1000 r.p.m. for 4 min. If some light flakes of Cu_2O persist in floating after centrifuging, add 2 to 4 drops of n-butyl alcohol to reduce the surface tension. Decant the excess Fehling's solution from the Cu_2O precipitate with a stream of water from a wash bottle, add 5 to 10 ml. of ferric ammonium sulphate solution, depending upon the amount of cuprous oxide present. Transfer the solution quantitatively to a 150-ml. beaker, add 6 to 9 drops of sodium diphenylamine indicator and titrate with standard potassium dichromate solution. At the end point, the color of the solution changes from an emerald green to an intense purple, and in practice it is not difficult to detect this change with ½ drop of potassium dichromate. Duplicate titrations should check within 0.05 ml.

Determination of sugar-copper ratios

Using the procedure outlined, the ratios of copper to glucose and invert sugar were determined. For this purpose stock solutions containing 5 mg. of sugar per milliliter were prepared from Pfanstiehl's highest purity glucose and sucrose, dried for 2 hours in a vacuum oven at 70° C. previous to weighing. Stock solutions of sucrose were hydrolyzed at pH 5.0 with 2 ml. of Difco invertase solution for 4 hours. The experimental curves showing the copper equivalents to glucose and invert sugar and glucose are shown

TABLE II

Invert sugar* $y = 0.4594x + 0.00028x^2$								
Copper	Sugar	0.1000N $K_2Cr_2O_7$	Copper	Sugar	0.1000N $K_2Cr_2O_7$	Copper	Sugar	0.1000N $K_2Cr_2O_7$
mg.	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*	*mg.*	*mg.*	*ml.*
5.0	2.30	0.77	85.0	41.06	13.31	165.0	83.43	25.85
10.0	4.66	1.57	90.0	43.61	14.10	170.0	86.18	26.68
15.0	6.90	2.38	95.0	46.17	14.90	175.0	88.96	27.25
20.0	9.30	3.14	100.0	48.74	15.70	180.0	91.76	28.21
25.0	11.64	3.93	105.0	51.32	16.49	185.0	94.61	29.00
30.0	14.03	4.71	110.0	53.93	17.25	190.0	97.41	29.80
35.0	16.42	5.50	115.0	56.53	18.05	195.0	101.2	30.62
40.0	18.83	6.28	120.0	59.16	18.84	200.0	113.1	31.42
45.0	20.24	7.07	125.0	61.80	19.60			
50.0	23.67	7.85	130.0	64.44	20.38			
55.0	26.11	8.62	135.0	67.11	21.19			
60.0	28.58	9.40	140.0	69.80	21.98			
65.0	31.04	10.20	145.0	72.61	22.75			
70.0	33.53	10.80	150.0	75.22	23.50			
75.0	36.03	11.74	155.0	77.93	24.25			
80.0	38.56	12.51	160.0	80.67	25.08			

* See footnote at the bottom of table I.

in figures 2 and 3. The sugar values for both invert sugar and glucose were calculated from the experimental data by the method of least squares and are shown in tables I and II.

Summary

1. A semi-micro modification of the MUNSON-WALKER-BERTRAND method for determining reducing sugars has been presented.

2. The method described uses smaller quantities of reagents, substitutes a water bath for digestion, centrifuges the cuprous oxide precipitate, and uses potassium dichromate as a standard oxidant.

The authors wish to acknowledge their indebtedness to Dr. W. M. ATWOOD for his helpful suggestions and for the use of his laboratory, and to Dr. W. E. MILNE for his suggestions on the calculation of the equations for the sugar curves.

DEPARTMENTS OF HORTICULTURE AND BOTANY
OREGON STATE COLLEGE

LITERATURE CITED

1. BERTRAND, G. Le dosage des sucres reducteurs. Bull. Soc. Chim., ser. 3, **35**: 1285–1299. 1906.
2. ILJIN, W. S. Bestimmung des Zuckers mittels Fehlingscher Lösung und Zentrifugierens. Biochem. Zeit. **193**: 322–325. 1928.
3. MUNSON, L. S., and THOMAS, P. H. The unification of reducing sugar methods. Jour. Amer. Chem. Soc. **28**: 663–686. 1906.
4. PHILLIPS, T. G. The determination of sugars in plant extracts. Jour. Biol. Chem. **95**: 735–742. 1932.
5. QUISUMBING, F. A., and THOMAS, A. W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved in current methods. Jour. Amer. Chem. Soc. **43**: 1503–1526. 1921.
6. WILLARD, H. H., and FURMAN, N. H. Elementary Quantitative Analysis. 1933.
7. SHAFFER, P. A., and SOMOGYI, M. Copper-iodometric reagents for sugar determinations. Jour. Biol. Chem. **100**: 695–713. 1933.

SAND CULTURE STUDIES OF THE USE OF SALINE AND ALKALINE WATERS IN GREENHOUSES

V. G. HELLER, R. H. HAGEMAN AND E. L. HARTMAN

(WITH FOUR FIGURES)

Introduction

Saline and alkaline waters, which are widely distributed over the earth's surface and are frequently found in the southwestern portion of the United States, are responsible for many troubles encountered in greenhouse production. The contamination comes from deep wells, salt beds, gypsum deposits, and alkaline planes, and is primarily composed of sodium, calcium, and magnesium chlorides, sulphates, or bicarbonates. Frequently the concentration of these waters approaches that of sea water. The use of such waters for plants is often disastrous.

The action of these salts occurring singly or in combination has frequently been discussed in connection with field irrigation. BREAZEALE of Arizona, LIPMAN of California, and HARRIS of Utah and their associates are especially deserving of credit for their investigations. Results of these field trials, however, cannot be safely applied to greenhouse problems, for here we find more frequent applications of water, more rapid evaporation, shallow beds, and a continuous concentrated production.

The effect and toxic levels of the various alkaline salts on greenhouse plants, as shown by accumulation of the salts in the soil has been reported by this station (6). The purpose of this experiment was to make a similar study of the effect of sodium chloride and sodium bicarbonate without accumulation of the salt in the soil solution.

Experimental

Comparable four-inch plants of Marglobe tomatoes were transferred to pots filled with silica and watered with a nutrient solution consisting of:

139.5 ml. of 0.5 mol. $MgSO_4$
139.5 " " " " KH_2PO_4
174.4 " " " " $CaNO_3$
15.2 " iron citrate soln. (4 gm. per 100 ml.)
15.2 " A–Z soln. as suggested by HAAS (5)

This mixture was diluted to four gallons with rain water and the desired kind and quantity of salt was added to make the respective solutions. pH determinations were made on each solution after the salts were added. The plants were watered frequently enough to provide for water requirements. Every seven days all pots were leached to prevent accumulation, and to

maintain a nearly constant concentration of salt. (Analysis of the sands at the close of the experiment proved little or no accumulation had occurred). Immediately after leaching, the plants were again watered with their respective solutions.

Daily records were made of the amount of solution used for each lot of five, the general appearance as to growth, the quantity and color of foliage, the length of life, and, finally, the average dry weight. At the completion of the tests, all plants were clipped at the ground level, dried, ground, and analyzed according to the methods of the Association of Official Agricultural Chemists. The results from the tomatoes receiving sodium chloride and sodium bicarbonate solutions varying from 100 to 3000 parts per million in concentration are presented in the accompanying figures.

Results and discussion

The plants in both series were uniform in appearance and in excellent condition at the start. Growth was rapid during the first week and no injuries were observed. Thereafter, injuries in the form of stunted growth and yellowed and dead leaves were noted in the higher concentrations. These injuries moved progressively to the lower salt concentrations as the experiment was continued. No sharp demarcation of injury could be detected, but a gradual increase with increased salt concentration was noticeable.

Injury was not as characteristic, and appeared more slowly, in the sodium chloride series than in the sodium bicarbonate. Chlorotic leaves appeared at the end of the third week on plants receiving concentrations of sodium chloride greater than 2500 parts per million. At the termination of the experiment, all plants treated with more than 1500 parts per million sodium chloride developed turgid, yellowish leaves which curled and then died. These conditions were very pronounced in the higher concentrations, 2300 to 3000 parts per million. BASLAVSKAJA (1) observed similar conditions in the leaves of potato plants treated with chloride salts and attributed them to the chloride ion. Plants receiving over 2000 parts per million of salt in both series were much more resistant to wilting, and their total water consumption was reduced one-third or more for the duration of the experiment.

When the experiment was terminated, observations indicated that salt injuries were apparent in concentrations as low as 900 parts per million of sodium bicarbonate and 1100 parts per million of sodium chloride. General condition of the plants indicated that concentrations greater than 1600 parts per million of sodium bicarbonate and 1700 parts per million of sodium chloride were toxic.

Data for the total growth of plants, represented by wet and dry weights of each group of five plants, are given in figures 1 and 2 for the sodium chloride and sodium bicarbonate series. In the sodium chloride series, the

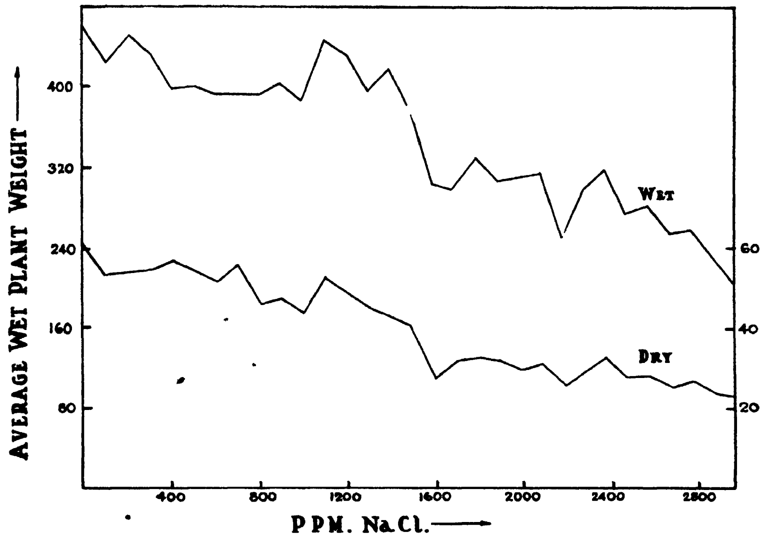


FIG. 1. Growth curves for tomato plants in sand cultures with NaCl in the nutrient solutions. Upper curve constructed on wet weights; lower curve on dry weights.

green weight of plants receiving over 2900 parts per million of the salt was one-half that of the checks. A similar retardation of growth as shown by its dry weight occurred at a concentration of 1600 parts per million sodium chloride. Since the plants which received over 2000 parts per million salt

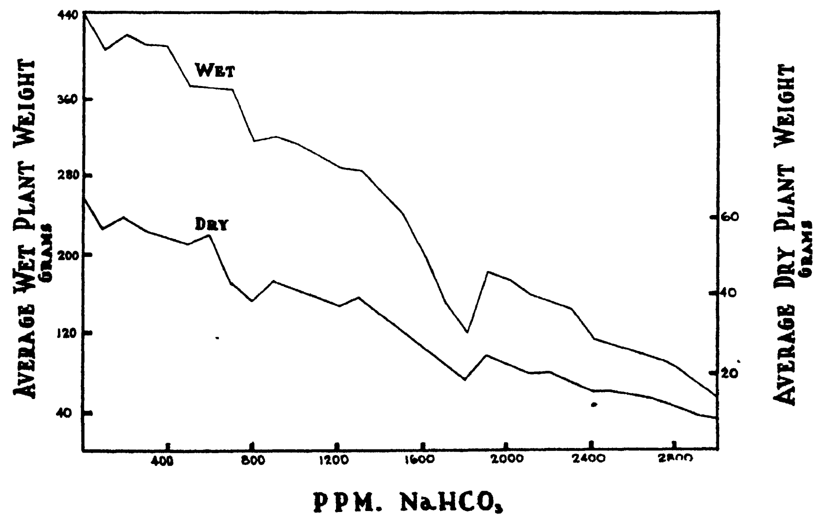


FIG. 2. Growth curves of tomato plants in sand culture with $NaHCO_3$ in the culture solutions.

had an increased moisture content of 3 to 3.5 per cent. more than the checks, the injury based on dry weights is the better criterion. In the sodium bicarbonate series, plants watered with over 1600 parts per million had a green weight one-half that of the checks. On a similar comparative basis of dry weights the concentration was 1400 parts per million. In the latter series the increase in the moisture content of the plants was not as pronounced. The green and dry weights of the checks were larger in all cases than those of the plants receiving the salt water treatment. BREAZEALE's (2) work with wheat seedlings treated with sodium chloride shows this same gradual declination in growth as concentration is increased.

Observations failed to detect any reduction in growth in the lower concentrations. The groups receiving salt concentrations lower than 600 parts per million appeared to be equivalent to or better than the checks. This is not in accord with LIPMAN's (7) statement that small concentrations of about 500 to 1000 parts per million sodium chloride may depress growth in early stages, but that higher concentrations may stimulate it.

Analysis of data presented in figures 3 and 4 shows many marked changes

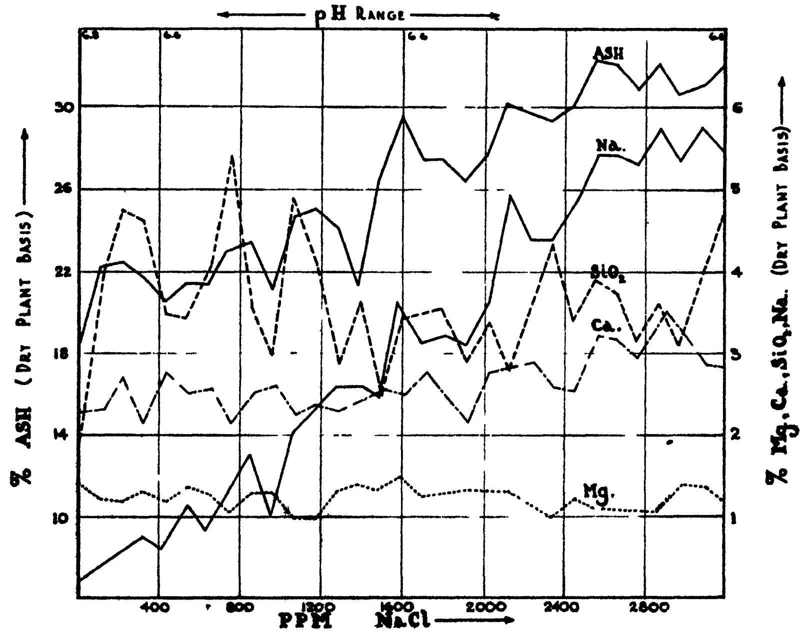


FIG. 3. Ash content of tomato plants grown in sand cultures with additions of NaCl in the nutrient solutions. Dry weight basis.

in the plants as they were subjected to increased concentrations of salts.

The ash content of plants treated with sodium chloride increased very rapidly and reached values as high as 32 per cent. of the dry plant weight.

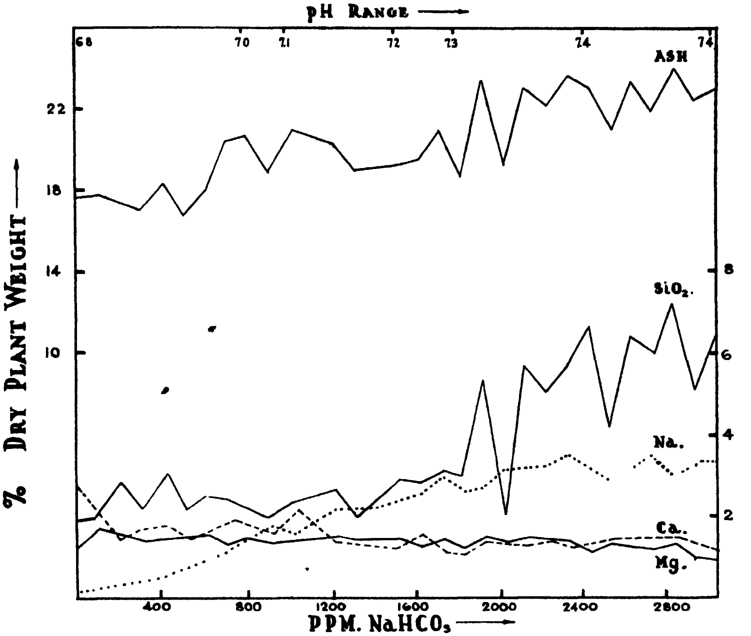


FIG. 4. Ash content of tomato plants grown in sand cultures with additions of $NaHCO_3$ to the nutrient solutions. Dry weight basis.

The addition of 100 parts per million sodium chloride to the solutions increased the ash content 4 per cent. of dry plant weight over the checks, and 1500 parts per million sodium chloride gave an increase of 11 per cent. Plants treated with concentrations of sodium chloride between 1300 and 1500 parts per million showed the most rapid increase in the ash content. The ash content of the sodium bicarbonate series did not increase as rapidly as that of the sodium chloride series, and did not reach exceedingly high values. Plants receiving from 500 to 800 parts per million sodium bicarbonate had a rather marked increase in ash.

The sodium content of plants treated with the higher concentrations of sodium chloride was over twenty times that of the checks. Plants receiving solutions containing between 1800 and 2000 parts per million of sodium chloride showed a very marked increase of sodium from 2.5 to nearly 5.0 per cent. of the dry plant weight. Although determination of the chloride ion was not made, it would be logical to assume that the sodium chloride content was increased. BUFFUM and SLOSSON (3), however, reported that in alfalfa plants the sodium chloride content decreased as the sodium chloride concentration was increased in the solution. The sodium content of the sodium bicarbonate series increased gradually. The higher concentrations of

sodium bicarbonate caused an increase of the sodium content to fifteen times that of the checks.

In explaining the larger ash and sodium content of the sodium chloride over the sodium bicarbonate series, it should be remembered that the higher conductance of the combined ions of sodium and chloride over those of sodium and bicarbonate would give them greater mobility which should tend to increase their diffusion into the plant tissue. The addition of sodium bicarbonate also increased the pH values, which would promote formation of insoluble compounds of iron, calcium, magnesium, phosphate, and carbonate which would tend to lower the ash content of these plants.

The calcium content of plants in the sodium chloride series, although quite irregular, is in general higher than in the check plants. In the sodium bicarbonate series, there is a rapid decrease in the calcium content up to a concentration of 200 parts per million. The calcium content remains nearly constant at values of at least one-fourth below the checks until a concentration of 2800 parts per million has been reached, at which point it undergoes a marked decrease to approximately one-half that of the checks.

The magnesium content of the plants treated with sodium chloride showed little change throughout the whole series. In the sodium bicarbonate series there was an increase in magnesium in the lower concentrations up to 600 parts per million. There was a rather gradual decline in the magnesium content of the plants from 600 to 3000 parts per million of sodium bicarbonate.

The increase in the magnesium and the decline in the calcium content upon the addition of 100 and 200 parts per million of sodium bicarbonate could possibly be explained by the fact that calcium carbonate is more insoluble than magnesium carbonate, and that under these conditions magnesium is absorbed to replace the unavailable calcium.

The increase in the calcium and the decrease in magnesium content of the plants receiving sodium chloride solutions is in agreement with the work of CAROLUS (4) on the absorption of these bases in the presence of sodium. The irregular trend of the silica content in the plants watered with sodium chloride solutions prevents it from giving any useful information. Plants treated with sodium bicarbonate solutions, however, showed a pronounced increase in silica content after a concentration of 1800 parts per million had been reached. It is interesting to note that at this concentration the pH has been raised to a value of 7.3. This increased pH would tend to render the silica soluble as sodium silicate, and thereby account for the increased silica content.

Summary

The conclusions to be drawn from the sand culture experiment where sodium chloride and sodium bicarbonate in varied concentrations from 100

to 3000 parts per million were added to tomatoes may be summarized as follows:

1. The data indicate that injuries were gradually accentuated as concentrations were increased. The sodium bicarbonate produced injuries at lower concentrations, and, in general, was more toxic than the sodium chloride.

2. Under the conditions used, concentrations of either salt above the range 1400–1600 parts per million proved to be extremely toxic.

3. The ash and sodium content of the plants increased more rapidly and reached higher values in the plants treated with sodium chloride than in those treated with sodium bicarbonate.

4. Treatment with sodium bicarbonate reduced the calcium content very markedly from one-fourth to one-half that of the checks.

5. Plants receiving sodium chloride developed characteristic turgid, yellow leaves that curled and died.

OKLAHOMA AGRICULTURAL EXPERIMENT STATION
STILLWATER, OKLAHOMA

LITERATURE CITED

1. BASLAVSKAJA, S. S., and SYROESHKINA, S. M. Influence of the chloride ion on the content of chlorophyll in the leaves of the potatoes. Plant Physiol. **11**: 149–157. 1936.
2. BREAZEALE, J. F. A study of the toxicity of salines that occur in black alkali soils. Arizona Agr. Exp. Sta. Tech. Bull. 14. 1927.
3. BUFFUM, B. C., and SLOSSON, E. E. Alkali studies V. Wyoming Agr. Exp. Sta. 10th Ann. Report. 1–15. 1900.
4. CAROLUS, R. L. Effect of certain ions, used singly and in combination on the growth and the postassium, calcium and magnesium absorption of the bean plant. Plant Physiol. **13**: 349–363. 1938.
5. HAAS, A. R. C., and REED, H. S. Significance of traces of elements not ordinarily added to culture solutions for the growth of young orange trees. Bot. Gaz. **83**: 77–84. 1927.
6. HELLER, V. G., HAGEMAN, R. H., and HARTMAN, E. L.. Effect of saline and alkaline waters upon greenhouse plants. Oklahoma Acad. Sci. **20**. 1940.
7. LIPMAN, C. B., DAVIS, A. R., and WEST, E. S. The tolerance of plants for NaCl. Soil Sci. **22**: 303–322. 1926.

INFLUENCE OF AUXINS ON REPRODUCTION OF *LEMNA MAJOR*

NORMAN ASHWELL CLARK AND ELMER E. FRAHM

(WITH ONE FIGURE)

At the present time there exists a large amount of evidence showing that auxins control many of the functions of green plants. Particular attention, from both qualitative and quantitative standpoints, has been paid by investigators to the effect of the presence of auxins in the plant on the elongation of cells, the growth of roots, and the increase in the vegetative parts (**1**, **8**, **15**).

The addition of prepared auxin material, supplied in various forms and in various ways, also has a marked effect on plants. ZIMMERMAN and WILCOXON (**16**) found that adventitious roots were initiated and stems and leaves were affected. THIMANN and LANE (**13**) have shown that in Avena and Triticum the addition of auxin increased the dry weight, accelerated vegetative growth, and slightly hastened flowering. LOEHWING and BAUGUESS (**9**) applied heteroauxin to the soil as well as to the plants and produced variation in growth.

There are also indications that cell division and reproduction in plants are influenced by different auxins. JOST in 1935 (**7**) investigated the effect of several growth-promoting substances on cell division. Recently PRATT (**11**) checked the influence of three synthetic chemicals on the reproduction of the green alga, *Chlorella vulgaris*. He found that indole-3-acetic acid (heteroauxin) stimulated the reproduction of the Chlorella cells when present in the nutrient solution at a concentration of 10 p.p.m. and gave increased stimulation at 50 p.p.m. Indole-3-n-propionic acid was less stimulating, but was in turn greater than indole-3-n-butyric acid. Several other carbon compounds, including allantoin, were tried, but none caused an increase in the number of cells comparable with the three indole acids.

In these laboratories the writers have used *Lemna major* (*Spirodela polyrhiza*) as the test plant. Lemna is a water plant capable of producing flowers but usually propagating asexually. Its culture lends itself to a control as rigid as can be obtained with Chlorella. For several years it has been grown in inorganic nutrient solutions without organic matter, free from all microörganisms, and at a definite temperature both under electric light and in sunlight. When the logarithm of the number of fronds at any time, t, is plotted against the number of days, the result is a straight line which is reproducible under the same conditions. The slope of the line, K, is a measure of the speed of reproduction. Flasks can be subcultured indefinitely, and effects of variations in pH (**3**), light (**2**), organic or inorganic materials (**5**) are shown in the slope of the curve if the rate of reproduction is

changed, and in the form or size of the fronds if the vegetative material is affected.

The effects of three synthetic compounds on Lemna were investigated and are reported here: indole-3-acetic acid (heteroauxin), phenylacetic acid, and phenylpropionic acid.[1] Phenylacetic acid was tested at the Boyce Thompson Institute by HITCHCOCK and ZIMMERMAN (6) and more recently by THIMANN and SCHNEIDER (14) in studying the relative activities of different auxins.

The general technique for the growth of the Lemna has been given previously. The plants, free from microörganisms for hundreds of generations, were grown in an inorganic solution of purified salts in Erlenmeyer flasks at 25° C. ± 0.5° C. Solutions were sterilized at 20 lb. pressure for 15 min. and the test material was added either before sterilization or after passing through a Pasteur-Chamberland filter. The results from the two methods of sterilizing test materials indicated heat stability at the low concentrations used. The plants, after growing in the solutions, were checked each week for freedom from microörganisms and if contaminated, the culture was discarded.

The pH of the inorganic solutions was 4.7 to 4.8. This is the optimum pH for Lemna reproduction under the conditions used (3). SKOOG (12) found that plants made a measurable absorption of auxin from low concentrations in solutions at or below pH 5. The addition of small amounts of organic material did not change the pH of the Lemna cultures appreciably.

Light was supplied by electric lamps for 14½ hours daily at 400 to 500 foot-candles intensity. As the light varied somewhat in different experiments owing to the age of the electric bulbs, the rate of reproduction, K, of the plants varied slightly. Two or three cultures as controls were included in each experiment, and consisted of the Lemna in the standard inorganic medium. In the tables K is the slope of the curve multiplied by 100, and indicates the speed of reproduction.

In the first series, water solutions of the synthetic compounds were sterilized by passage through a Pasteur-Chamberland filter and were added, under sterile conditions, to the autoclaved inorganic solutions. The Lemna plants were transferred twice a week to fresh media containing the auxins.

At high concentrations (100 mg. per liter of media) all three compounds were toxic, and the Lemna died. At 10 mg. per liter both indole-3-acetic and phenylacetic also killed the plants, but phenylpropionic at that concentration produced small and light-colored fronds, with roots reduced to short stubs. At 1 mg. per liter, the rate of reproduction was slow and there was a marked shortening of the roots with all three acids; but the vegetative

[1] We are indebted to Dr. R. H. MANSKE of the National Research Council at Ottawa Canada, for the indole-3-acetic acid; the other two are commercial preparations.

characters varied. The indole-3-acetic produced small fronds, light green, with long strands connecting mother and daughter fronds. On the other hand, the phenylacetic increased the size of the fronds, and the color compared to the standards, was darker. Phenylpropionic at this concentration had no effect on either size or color. Similar results were obtained with the concentration at 0.1 mg. per liter.

The changes caused by these last two concentrations were not permanent and the cultures recovered when transferred to the standard inorganic medium. In 4 weeks the fronds appeared normal, with roots of the usual size and reproduction approached the regular rate. Cultures from phenylacetic and phenylpropionic acids, when recovering in the standard solutions, produced fronds of large size, but these did not reproduce large daughter fronds.

Table I shows the compounds and their concentration from 0.01 to 0.0001 mg. per liter of media, the rate of reproduction K, and the effect on the fronds. The plants in the inorganic control solutions without additions are described as normal. These fronds are of good size (somewhat larger than usually found in ponds), with many well-developed roots, and have a deep green color and healthy appearance. The average of duplicates is given; K usually varied only 0.1 to 0.2.

TABLE I

RATE OF REPRODUCTION OF LEMNA WITH ADDITIONS OF AUXINS TO THE MEDIA

COMPOUND	CONCENTRATION	K	CONDITION OF PLANTS
	mg./liter of media		
(Control)	0.00	8.5	normal
Indole-3-acetic	0.01	8.6	fronds slightly lighter green, roots somewhat shorter
	0.001	8.4	normal
	0.0001	8.5	"
Phenylacetic	0.01	8.8	normal
	0.001	9.0	"
	0.0001	8.8	"
Phenylpropionic	0.01	8.9	roots slightly shorter
	0.001	9.0	normal
	0.0001	8.7	"

With these low concentrations there was little or no increase in the rate of reproduction, and as little effect on the fronds. The 0.01 mg. per liter concentrations of indole-3-acetic acid and phenylpropionic caused some inhibition of root growth, but this effect was serious only at higher concentrations. Toxic properties of the indole-3-acetic acid began to show at 0.1 mg. per liter. PRATT (11) found his maximum increase in reproduction of

algae at 50 mg. per liter. The difference may lie in the effect on the root system.

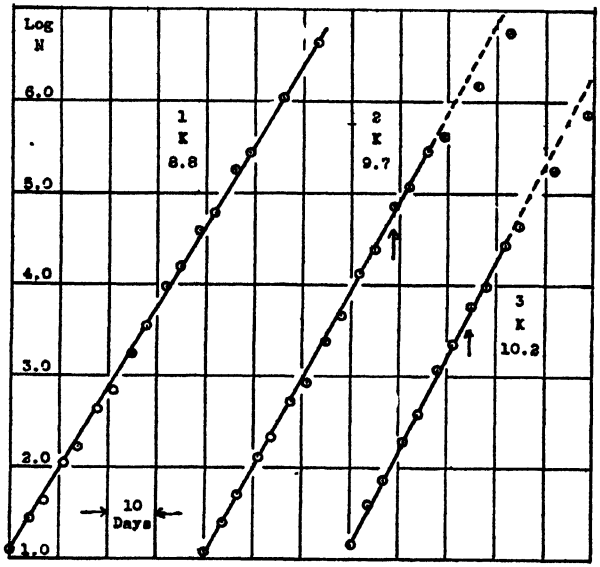


FIG. 1. Log. of number of plants on days
1. Standard control. K (slope × 100) = 8.8
2. Intermittent treatment with indole-3 acetic acid. $K = 9.7$
3. Intermittent treatment with phenylacetic acid. $K = 10.3$
↑ Treatment discontinued.

MACHT and GRUMBEIN (10) reported a definite stimulation of the roots of *Lupinus albus* seedlings after short exposure to very dilute solutions of beta-indolyl acetic acid. With the Lemna, it was observed that plants which had been treated with phenylacetic acid and phenylpropionic acid increased in size when placed in standard solutions. A further experiment was therefore planned in which the same three compounds were applied intermittently to the plants.

In this second series the Lemna plants were transferred each week to fresh standard media and the plants counted and checked for sterility as before. Each day, excluding Sundays, the plants in all cultures, except the controls, were transferred aseptically to solutions of the synthetic compounds, which were sterilized by passing through Pasteur-Chamberland filters. The Lemna remained in the solutions for 30 minutes on the laboratory table and were then returned to the standard salt solutions.

Table II shows the concentrations of the solutions, the rate of reproduction K, and the condition of the plants. The Lemna in the inorganic control solutions, without additions, are again described as normal and the test plants compared with these. Duration of the experiment was 42 days.

TABLE II

EFFECT OF INTERMITTENT APPLICATION OF AUXINS ON LEMNA

COMPOUND	CONCENTRATION	K	CONDITION OF PLANTS
	mg./liter media		
(Control)	0.00	8.6	normal
Indole 3-acetic	10.0	8.5	small fronds, short roots
	2.0	9.0	normal
	1.0	9.6	"
	0.5	9.5	"
	0.1	9.5	"
Phenylacetic	10.0	8.5	"
	5.0	9.5	"
	1.0	10.0	"
	0.1	10.6	fronds normal, root length increased
Phenylpropionic	10.0	10.0	normal
	1.0	10.0	"
	0.1	10.6	"

The periodic application produced marked increases in the rate of reproduction at concentrations which were toxic when the auxins were mixed with the standard medium. There is a greater difference than expected, especially in view of the fact that SKOOG (**12**) found that the activity of the indole-3-acetic decreased rapidly in a nutrient solution. He found about half the activity disappeared in 10 hours and almost all in 24 hours whether plants were present or absent.

A final experiment was carried out in order to check the results of this intermittent application and to compare the sterilization of the auxin solutions by the Pasteur-Chamberland filter and by steam sterilization. The same technique was used as in the second series, and two cultures were included with the control solutions (one of which was spoiled) in which the fronds were removed from the standard media once a day and placed in 100 ml. of sterile distilled water for 30 minutes, at the same time as the test plants were in the auxin solutions. Only one concentration, 1 mg. per liter, was used for the three auxins. The plants varied very little, if any, from the normal except those given the distilled water treatment, which were distinctly lighter in color. Table III gives the treatment and the rate of reproduction.

As noted previously, no differences in rates of reproduction were found when the auxin solutions were sterilized by autoclaving and by filtration. In both table II and table III, phenylpropionic acid is as effective as phenylacetic acid, and both stimulate reproduction of the Lemna more than the indole-3-acetic acid.

TABLE III

RATE OF REPRODUCTION OF LEMNA WITH INTERMITTENT AUXIN APPLICATION
CONCENTRATION 1 MG. PER LITER
STERILIZATION BY FILTRATION OR AUTOCLAVE

COMPOUND	TREATMENT	K
(Control)	autoclaved	8.8
"	"	9.0
Standard solution—with 30 minute water treatment	autoclaved	9.0
Indole-3-acetic acid	filtered	9.7
" " " "	autoclaved	9.6
Phenylacetic acid	filtered	10.1
" "	autoclaved	10.2
Phenylpropionic acid	filtered	10.3
" "	autoclaved	10.2

When the treatments shown in table III were discontinued, the rate of reproduction gradually dropped. Figure 1 shows three growth curves from table III; the logarithm of the number of fronds is plotted on time in days. Curve 1 is the control, $K = 8.8$; curve 2 is the result of treatment with 1 mg. of indole-3-acetic acid; and curve 3 with 1 mg. of phenylacetic acid. In the last two cases the drop in the rate of reproduction is shown when the treatment was stopped.

Summary

1. Indole-3-acetic acid, phenylacetic acid, and phenylpropionic acid were tested in varying concentrations for their effect on the rate of reproduction of *Lemna major*.

2. The Lemna was grown in inorganic solutions, under controlled light and temperature, free from microörganisms.

3. When added to the media twice weekly, the sterilized auxins failed to increase the reproduction markedly at concentrations of 0.01 mg. per liter or lower, and higher concentrations produced more or less toxicity. Ten milligrams per liter or slightly above killed the plants.

4. Intermittent application of the auxins produced increased reproduction. Both phenylacetic acid and phenylpropionic acid were more effective than indole-3-acetic acid.

5. Lemna which had been subjected to the influence of the auxins, returned to the normal rate of reproduction when replaced in the standard inorganic medium.

THE DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE

LITERATURE CITED

1. BOYSEN JENSEN, P. Growth-hormones in plants. Trans. AVERY, G. S., and BURKHOLDER, P. R. New York. 1936.
2. CLARK, N. A. The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. **29**: 935–941. 1925.
3. ———. Plant growth-promoting substances, hydrogen ion concentration, and the reproduction of Lemna. Plant Physiol. **1**: 273–279. 1926.
4. ———. Technique for the growth of Lemna under sterile conditions with controlled temperature and light. Iowa State Coll. Jour. Sci. **7**: 13–16. 1932.
5. ———, and ROLLER, E. M. The stimulation of *Lemna major* by organic matter under sterile and non-sterile conditions. Soil Sci. **31**: 299–309. 1931.
6. HITCHCOCK, A. E., and ZIMMERMAN, P. W. Effect of growth substances on the rooting response of cuttings. Contrib. Boyce Thompson Inst. **8**: 63–79. 1936.
7. JOST, L. Wuchstoff und Zellteilung. Ber. d. bot. Ges. **53**: 733–750. 1935.
8. KÖGL, F., HAAGEN-SMIT, A. J., und ERXLEBEN, H. Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Graskoleoptilen. Zeitschr. physiol. Chem. **228**: 104–112. 1934.
9. LOEHWING, W. F., and BAUGUESS, L. C. Plant growth effects of heteroauxin applied to soil and plants. Science n.s. **84**: 46–47. 1936.
10. MACHT, D. I., and GRUMBEIN, M. L. Influence of indole-acetic, indolebutyric, and naphthalene acetic acids on roots of *Lupinus albus* seedlings. Amer. Jour. Bot. **24**: 457–460. 1937.
11. PRATT, R. Influence of auxins on the growth of *Chlorella vulgaris*. Amer. Jour. Bot. **25**: 498–501. 1938.
12. SKOOG, F. Absorption and translocation of auxin. Amer. Jour. Bot. **25**: 361–372. 1938.
13. THIMANN, K. V., and LANE, R. H. After-effects of the treatment of seed with auxin. Amer. Jour. Bot. **25**: 535–543. 1938.
14. ———, and SCHNEIDER, C. L. The relative activities of different auxins. Amer. Jour. Bot. **26**: 328–333. 1939.
15. WENT, F. W., and THIMANN, K. V. Phytohormones. New York. 1937.
16. ZIMMERMAN, P. W., and WILCOXON, F. Several chemical growth substances which cause initiation of roots and other responses in plants. Contrib. Boyce Thompson Inst. **7**: 209–229. 1935.

AN ESTIMATION OF THE VOLUME OF WATER MADE AVAILABLE BY ROOT EXTENSION

PAUL J. KRAMER AND T. S. COILE

Modern views on plant-soil moisture relations stress the importance of continuous elongation of roots into new regions of the soil as an important factor in making water available to plants. It was long believed that as roots absorb the available water from the soil particles with which they are in direct contact more water is made available by capillary movement from more distant soil particles. Investigations conducted during the past 15 years indicate that this belief is not true, capillary movement of water toward the roots being so slow under average field conditions that it is of negligible importance. A brief discussion of soil-water relations will show why capillary movement is relatively unimportant.

If a limited amount of water is applied to a large volume of soil a part of the soil will be wetted uniformly while the remainder is unaffected. This situation is often observed after a shower which has wetted the upper three or four inches of soil, leaving a sharp line of demarcation between the moist soil and the dry soil beneath. The movement of water in a dry soil can occur only from larger to smaller capillaries. As PURI (6) has indicated, water will move from a wet soil to a dry soil only if the dry soil contains some capillaries smaller than the largest ones in the wet soil which are full of water. Water movement from wet soil to dry soil continues until contact is broken between the smaller capillaries of the dry soil which are responsible for the movement. When continuity of the liquid phase is thus broken visible movement of water ceases. The swelling of certain types of soil colloids when wetted is also of importance in inhibiting water movement by reducing the size of capillaries to a diameter so small that water molecules cannot readily pass. The amount of water held by capillarity in the soil after it has been uniformly distributed by gravitational and capillary forces is usually termed the field capacity (VEIHMEYER and HENDRICKSON, 10). Under field conditions well drained soils are usually assumed to be at their field capacity a day or two after being wetted by rain or irrigation. SHANTZ (7) stated that on theoretical grounds the capillary movement of water from moister to drier regions in soils at or below their field capacity would be very slow and this was experimentally verified by KEEN (3) and VEIHMEYER and HENDRICKSON (9). According to PURI (6) when soil moisture is restricted to capillaries formed by particles having a diameter of 0.001 mm. or smaller relatively little water is available to plants. He considers that particles coarser than clay are mainly responsible for retaining moisture available to plants, whereas clay aids in the conservation of

this moisture by reducing its rate of movement. LIVINGSTON (4), KEEN (3), SHULL (8), and others have pointed out that if little or no capillary water moves toward the roots then continual extension of the roots into new regions of the soil is essential to the absorption of an adequate supply of water.

While the importance of root extension in relation to absorption is becoming well recognized, no attempt has ever been made to show how much water is actually made available by the growth of roots through the soil. The recent investigation by DITTMER (1) of the number, size, and length of roots developed by winter rye (*Secale cereale* L.) supplied data on root extension which makes such calculations possible.

DITTMER reported that his rye plants produced an average of 3.1 miles of new roots per day during a period of four months. These roots were mostly 120 to 250 microns in diameter and covered with root hairs which were mostly 700 to 800 microns in length. It therefore seems safe to assume that these roots and root hairs contacted the soil particles in a cylinder of soil about 2 mm. in diameter and 3.1 miles long, having a total volume of approximately 15,700 ml. If the soil mass penetrated by the roots was at the field capacity a volume of water became available to the plants each day equal to the difference between the volume present at field capacity and the volume present at the wilting percentage. No data are available for the soil used by DITTMER but one of the writers has considerable data on volume-moisture relations for several types of soil.

The volume of water available for plants in two widely different textural classes of soils is illustrated by the data for a coarse sandy loam and a heavy clay soil. The mechanical composition of these soils is given in table I and the water-holding characteristics in table II. Table II gives the moisture

TABLE I

PHYSICAL CHARACTERISTICS OF SOILS

SOIL FRACTION AND PARTICLE SIZE CLASS	TEXTURAL CLASS	
	SANDY LOAM	CLAY
	%	%
Sand, 2.00 to 0.05 mm.	78.5	13.4
Silt, 0.05 to 0.002 mm.	14.5	27.7
Clay, 0.002 mm.	7.0	58.9
Total	100.0	100.0
"Total colloidal*	14.2	74.6
Percentage of organic matter on oven-dry basis	1.5	1.5
Volume-weight ratio	1.75	1.24

* BOUYOUCOS, G. J. Soil Sci. 26: 233–238. 1928, includes all the clay and a part of the silt.

TABLE II

THE WATER-HOLDING CHARACTERISTICS ON A VOLUME BASIS OF THE TWO SOILS

Textural class	Percentage of organic matter on oven-dry weight basis	Percentage of volume		Ml. of water available per liter of soil	Volume water available in 15,700 ml. soil
		Moisture equivalent	Wilting percentage		
	%	%	%	*ml.*	*ml.*
Sandy loam	1.5	15.0	4.8	102	1,601
Clay	1.5	46.0	27.5	185	2,904

equivalents and wilting percentages calculated on a volume basis, the volume of available water per liter of soil and the volume which would be available in the soil mass penetrated each day by the roots. The volume of available water is assumed to be the difference between the volumes of water at the wilting percentage and at the field capacity. There are 102 ml. of available water per liter of sandy loam soil and 185 ml. of available water per liter of clay soil. Assuming the addition of 3.1 miles of new roots per day, 1.6 liters of water would have been made available to the rye plant in the sandy loam soil and 2.9 liters in the clay soil. No information is available concerning the actual transpiration rate of the rye plant, but it had 50 square feet of leaf surface, including both sides, or less than one square meter if only one surface was considered. MILLER (5) reported that in July the daily transpiration per square meter of leaf surface was over 1300 ml. for corn and over 2000 ml. for milo. The transpiration rate of rye growing in the greenhouse in the winter was doubtless much lower and it therefore seems highly probable that the daily root elongation alone made available sufficient water to supply the requirements of the plant. It should also be remembered that 3.1 miles of roots was merely the average addition per day. Since only a few feet of roots were added during the first week the daily increase in length was much in excess of 3.1 miles during the latter part of the experiment.

Continuing with the assumption that 3.1 miles of new roots were added each day the volume of new soil occupied daily amounted to over 30 per cent. of the total volume of soil in which the plant was growing. It therefore seems obvious that during the latter part of this experiment the new roots were crossing and recrossing older roots and thus daily reoccupying the same soil mass. This idea is also supported by the fact that at the conclusion of the experiments the total volume of roots and root hairs appears to have amounted to over one-sixth of the volume of the soil mass in which they were growing. The total surface of roots and root hairs was estimated

to be 6,875.4 square feet. How much of this surface was in actual contact with soil particles is unknown. Since the number of root hairs ranged from 19 to 53 per mm. of root it seems probable that adequate contact was made with all soil particles lying within the zone occupied by the root hairs to insure absorption of all the available water.

It is realized that this is a somewhat hypothetical example. The number and length of roots and rate of growth probably would not be the same in a sandy loam soil and in a clay soil. In spite of their imperfections, however, these calculations emphasize the fact that root extension may make available considerable quantities of water. For every 100 feet of roots added to the rye plant about 9.5 ml. of water would become available in the sandy loam soil and about 17.7 ml. in the clay soil. DITTMER cited evidence indicating that other species of grasses may have equally extensive root systems. In a more recent study (**2**) he reported that a cubic inch of soil contained 630 feet of roots and root hairs of oats. A similar volume of soil contained over 4000 feet of roots and root hairs of Kentucky bluegrass while a cubic inch of soil under rye contained 1300 feet of roots and root hairs. Such complete penetration probably does not occur with many plants other than grasses. The problem of root growth as related to the absorption of water deserves further investigation. In future studies it would be desirable to obtain data concerning the water-holding characteristics of the soil and the transpiration rate of the plants so that the rôle of root extension in supplying water could be more accurately determined.

Summary

The volume of water made available daily by root growth was calculated for winter rye using the data published by DITTMER. It was assumed that the roots contacted all soil particles in a cylinder 2 mm. in diameter and that 3.1 miles of roots were added daily. This amount of root extension would make available about 1.6 liters of water daily in a sandy loam soil at field capacity and about 2.9 liters in a heavy clay soil. It appears that at least under some conditions root extension might supply all the water required by a plant.

DEPARTMENT OF BOTANY AND
SCHOOL OF FORESTRY
DUKE UNIVERSITY

LITERATURE CITED

1. DITTMER, H. J. A quantitative study of the roots and root hairs of a winter rye plant (*Secale cereale*). Amer. Jour. Bot. **24**: 417–420. 1937.

2. ———————. A comparative study of the subterranean members of three field grasses. Science, n. s. **88**: 482. 1938.
3. KEEN, B. A. The limited rôle of capillarity in supplying water to plant roots. Proc. First Int. Congress Soil Sci. **1**: 504–511. 1927.
4. LIVINGSTON, B. E. Plant water relations. Quarterly Rev. Biol. **2**: 494–515. 1927.
5. MILLER, E. C. Plant physiology. Second edition. McGraw-Hill. 1938.
6. PURI, A. N. Physical characteristics of soils: V. The capillary tube hypothesis of soil moisture. Soil Sci. **48**: 505–520. 1939.
7. SHANTZ, H. L. Drought resistance and soil moisture. Ecology **8**: 145–157. 1927.
8. SHULL, C. A. Absorption of water by plants and the forces involved. Jour. Amer. Soc. Agron. **22**: 459–471. 1930.
9. VEIHMEYER, F. J., and HENDRICKSON, A. H. Soil-moisture conditions in relation to plant growth. Plant Physiol. **2**: 71–82. 1927.
10. ——————— and ———————. The moisture equivalent as a measure of the field capacity of soils. Soil Sci. **32**: 181–193. 1931.

DETERMINATION OF THE NITROGEN FRACTIONS IN ATLAS AND MILO SORGHUM PLANTS[1]

JAMES E. WEBSTER AND HOWARD MITCHELL

(WITH ONE FIGURE)

Introduction

During the course of experiments conducted to find an explanation for the varying susceptibility of sorghum varieties to chinch bug injuries, considerable evidence has been collected indicating that nitrogen may be one of the factors involved in the problem. It is a known fact that, in general, additions of nitrogen fertilizers to plants increase the susceptibility of such plants to insect injury (**3**); DAHMS has confirmed this fact for sorghums (**1**). Our work on the chemical composition of various varieties has shown that susceptible varieties generally have a higher nitrogen content than non-susceptible varieties grown under similar conditions. Particularly is this true of field-grown Atlas and Dwarf Yellow Milo plants, which are representative, respectively, of highly resistant and extremely susceptible varieties. The study was undertaken to determine whether certain nitrogen fractions were present in greater abundance in one variety than in the other, such information being desired for cultural solution studies dealing with insect susceptibility of plants, and in the preparation of solutions for culturing chinch bugs.

Material and methods

Most workers now seem agreed that the use of fresh plant tissue is essential for study of the nitrogen fractions in plants, and this view has been adopted for our work. The Atlas and Milo plants were grown on a sandy soil in adjacent plots. The plants, which were grown at some distance from the laboratory, were cut at the surface of the ground and transported at once to the laboratory. Here they were immediately ground in a power meat grinder, and samples taken for fractionation, solids, and total nitrogen. The total time which elapsed between cutting and sampling in no instance exceeded one hour, and was often somewhat less. Large samples of plants were secured, varying from many hundreds of plants when small, to at least fifteen for samplings later in the season. Plants were cut at 12:30 P.M. except for one sample cut at 10:00 A.M. The methods employed were modifications of the procedure of DAVIDSON and SHIVE which are adapted to cyanogenetic plants (**2**).

Precautions were taken to prevent contamination by ammonia in the air,

[1] Published with the approval of the Director of the Oklahoma Agricultural Experiment Station.

and blanks were also run as checks on all determinations, to further eliminate this factor.

CYANOGENETIC NITROGEN.—Fifty-gram samples of the ground tissue were placed in liter Erlenmeyer flasks containing 200 ml. of boiling water, and aerated for three hours; the HCN was collected in 5 per cent. NaOH and estimated by titration with silver nitrate. The samples were then filtered, and the residues transferred to large mortars and ground for ten minutes, then boiled and filtered. The grindings, boilings, and filtrations were repeated until the combined extracts measured about 900 ml. The extracts were then heated to boiling, 1 to 2 ml. of 10 per cent. acetic acid added, and boiling continued for one minute. The extracts were again filtered and made up to one liter. Five-hundred-ml. aliquots of these extracts were adjusted to pH of 5.5–6.0, 0.05 gm. of emulsin added, the samples heated to 50° C., and aerated and titrated as before. The two values for cyanides were combined, and the results expressed as cyanogenetic nitrogen.

SOLUBLE NITROGEN.—Aliquots of the filtered solutions were evaporated nearly to dryness in Kjeldahl flasks, and the nitrogen estimated by the Kjeldahl-Gunning-Arnold method, modified to include the nitrogen of nitrates.

AMMONIA NITROGEN.—The cyanide-free solutions were made just alkaline to phenolphthalein, and aerated into standard acid for three hours.

AMIDE NITROGEN.—The ammonia-free extracts were neutralized, and enough H_2SO_4 added to make 5 per cent. solutions. These were then refluxed for two-and-one-half hours, made alkaline, and the ammonia removed by aeration as before.

HUMIN NITROGEN.—The amide-free solutions were neutralized, and then filtered using suction. After thorough washing, the precipitates were transferred to Kjeldahl flasks, and nitrogen determined by the Kjeldahl-Gunning-Arnold method. The filtrates were diluted to one liter.

BASIC NITROGEN.—One-hundred-ml. aliquots of the filtrates from the humin nitrogen determinations were acidified by the addition of 5 ml. of H_2SO_4, and 30 ml. of phosphotungstic acid precipitating solution. The samples were placed in a refrigerator for 24 hours, removed, filtered, and total nitrogen determined on the precipitates as in the humin fraction.

AMINO NITROGEN.—Aliquots of 700 ml. of the humin nitrogen filtrate were concentrated to 200 ml., and alpha amino nitrogen estimated, using the micro Van Slyke apparatus.

NITRATE NITROGEN.—Aliquots of 50 ml. were taken of the humin filtrates, diluted with 200 ml. of water, and nitrates estimated by the Devarda method.

TOTAL NITROGEN.—The Kjeldahl-Gunning-Arnold procedure was used on 10 gm. samples of the freshly ground tissues.

Data and discussion

The data collected are presented in table I and in figure 1. The age of

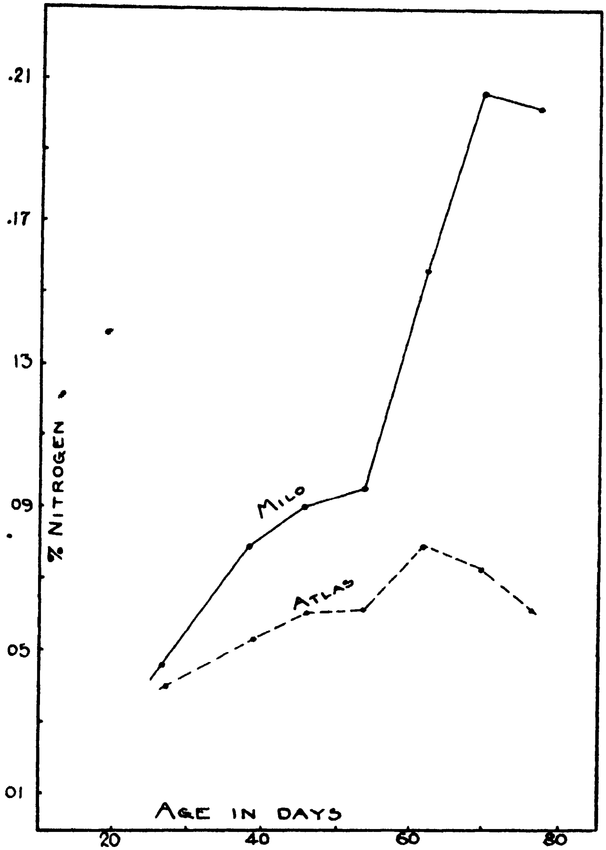


FIG. 1. Combined percentages of amino and basic nitrogen.

plants is expressed as days from planting, since the location of the field precluded daily observations to note when the shoots emerged. Attention is called to the fact that during the early stages of growth, Milo plants were larger and more vigorous than Atlas plants, and that it was not until after the third sampling (46 days) that Atlas plants exceeded the Milo plants in size, although Milo is called a dwarf variety and Atlas a tall variety.

SOLUBLE NITROGEN.—A major difference is noted here in that generally the soluble fraction in Milo increases as the plants age, while in Atlas this fraction fluctuates but little and at the last sampling reaches its lowest point. This is of interest, for it is the soluble fraction that would be expected to furnish the nitrogen available to the chinch bugs.

Cyanide, ammonia, humin, and nitrate nitrogen percentages, while varying some at specific dates and in total amounts, are all relatively low, and show the same general trends for both varieties.

TABLE I

NITROGEN FRACTIONATION IN SORGHUM PLANTS
FIELD GROWN (1939)

Age	Height	Solids	Nitrogen									
			Total	Insoluble	Soluble	Cyanide	NH_3	Amide	Humin	Basic	Amino	Nitrate
Milo												
days	in.	%	%	%	%	%	%	%	%	%	%	%
28	7	18.60	0.470	0.364	0.106	0.0054	0.0003	0.0000	0.0060	0.0140	0.0362	0.016
38	12	20.35	0.432	0.320	0.112	0.0041	0.0005	0.0003	0.0072	0.0404	0.0390	0.000
46	15	17.52	0.432	0.302	0.130	0.0054	0.0005	0.0000	0.0036	0.0445	0.0451	0.024
53*	20	17.48	0.412	0.291	0.121	0.0023	0.0001	0.0003	0.0116	0.0584	0.0360	0.032
61‡	29	18.02	0.419	0.248	0.171	0.0027	0.0003	0.0000	0.0056	0.0804	0.0753	0.028
69	36	21.64	0.442	0.252	0.190	0.0026	0.0013	0.0000	0.0032	0.0904	0.1153	0.008
76†	42	27.21	0.508	0.265	0.243	0.0026	0.0017	0.0000	0.0000	0.0624	0.1408	0.008
Atlas												
28	5	21.26	0.455	0.352	0.103	0.0072	0.0007	0.0030	0.0088	0.0090	0.0331	0.008
38	10	20.83	0.406	0.308	0.098	0.0042	0.0000	0.0055	0.0080	0.0304	0.0240	0.000
46	15	14.70	0.370	0.258	0.112	0.0032	0.0011	0.0000	0.0092	0.0340	0.0271	0.008
53*	28	13.10	0.322	0.210	0.112	0.0025	0.0005	0.0031	0.0056	0.0384	0.0240	0.020
61‡	42	12.50	0.279	0.176	0.103	0.0018	0.0013	0.0051	0.0024	0.0504	0.0295	0.012
69	50	15.32	0.290	0.195	0.095	0.0016	0.0011	0.0005	0.0056	0.0324	0.0404	0.008
76†	60	21.39	0.279	0.201	0.078	0.0007	0.0009	0.0014	0.0000	0.0280	0.0329	0.004

* Atlas plants now exceed Milo in diameter also.
† Milo fully headed; Atlas just beginning.
‡ Plants cut at 10:00 A.M.

Amide nitrogen is found more abundantly in Atlas plants; on the basis of the small amounts present, however, it is not considered significant.

Basic nitrogen percentages are much larger than those of the previous soluble fractions considered, and here we find that the Milo percentages are consistently higher, although showing the same seasonal trend that is found in Atlas plants.

Amino nitrogen percentages show by far the greatest varietal differences, and it is in this fraction, combined with the basic fraction (largely diamino acids) (**4**), that we see an explanation for the higher percentages of soluble nitrogen found in the Milo plants. This difference is more strikingly shown by referring to the graph, figure 1, where these two values for amino nitrogen have been combined. This shows not only the much greater percentages found in Milo plants, but also some differences in seasonal trends. It is thus apparent that, disregarding some small individual variations, most of the differences in soluble nitrogen content can be accounted for by variations in the amino nitrogen content.

Summary

Field grown Dwarf Yellow Milo plants are shown to be higher in nitrogen content than Atlas plants.

Soluble nitrogen generally increases in the growing Milo plants and remains constant or decreases in the Atlas plants.

Amide nitrogen is practically absent in growing milo plants.

Most of the observed differences in soluble nitrogen content between the Dwarf Yellow Milo plants (chinch bug susceptible), and Atlas (resistant) can be accounted for in the basic and alpha amino nitrogen fractions.

OKLAHOMA AGRICULTURAL EXPERIMENT STATION,
STILLWATER, OKLAHOMA

LITERATURE CITED

1. DAHMS, REYNOLD G. Personal communication to the authors. Assistant in Entomology, located at Lawton, Oklahoma.
2. DAVIDSON, O. W., and SHIVE, J. W. Determination of the nitrogenous fractions in vegetative tissue of the peach. Plant Physiol. **10**: 93–114. 1935.
3. MARTIN, HUBERT. The scientific principles of plant protection. 2nd ed., Edward Arnold and Co., London, 1936.
4. MURNEEK, A. E. Biochemical studies of photoperiodism in plants. Missouri Agr. Exp. Sta. Res. Bull. 268. 1937.

SOME EFFECTS OF STANNOUS SULPHATE AND STANNIC CHLORIDE ON SEVERAL HERBACEOUS PLANTS

BARNEY BARNETT COHEN

(WITH THREE FIGURES)

Introduction

Until recently it was thought that boron, manganese, copper, zinc (4), and iron (7) were the only essential trace elements necessary for plant growth. Investigators now believe that other elements also are essential. YOUNG (14) observed that additions of rare elements, including tin, stimulated the growth of timothy and algae. ARNON (2) suggested that such elements as Mo, V, Ti, W, Cr, Cd, and Co are essential to maximum growth of lettuce and asparagus. MICHEELS (9) reported that solutions of colloidal tin had a marked effect upon the early growth of wheat, oats, peas, and buckwheat. ALLISON, HUNTER, and BRYAN (1) noted slight favorable responses with additions of tin and other elements in sixty crop plants grown on the peat soils of the Florida everglades. In growing wheat, rye, maize, and tobacco in water culture SCHROPP and SCHARRER (11) found that traces of tin chloride with other elements induced favorable responses.

In the experiments reported here, stannous sulphate was added to nutrient solutions and observations were made of its effects upon corn and peas. The effects of varying concentrations of stannic chloride were observed for the sunflower only.

Procedures

Sunflowers, corn, and garden peas were grown in glazed pots, six plants to each pot. Quartz sand was used as the growth medium. The cultures were supplied with nutrient solutions twice daily, and flushed twice weekly with distilled water to prevent accumulation of salts in the sand. Solutions were prepared from half-molar stock solutions of calcium nitrate, dipotassium phosphate, and magnesium sulphate, purified as first suggested by STEINBERG (12) with the modifications adopted by ARNON and STOUT (3). Calcium carbonate (65 gm.) was added to 5 liters of stock solution, which was then autoclaved for 20 min. at 15 lb. (120.5° C.). Nutrient solutions were made from the purified stocks by diluting 12 ml. of calcium nitrate, 9 ml. of dipotassium phosphate, and 9 ml. magnesium sulphate with distilled water (copper still) to 1 liter. In addition to the trace elements, Cu, B, Mn, and Fe, tin in the form of stannous sulphate was supplied to the nutrient in concentrations of 0.2, 1, 5, 20, and 100 p.p.m. In the sunflower tests, tin was added as stannic chloride in concentrations of 0.01, 0.05, 0.1, 0.5, 1, 10, and 100 p.p.m. No attempt was made to purify the trace element solu-

tions since these were present only in minute quantities. Stock solutions were brought to pH 6.5; and to prevent contamination, glassware was cleaned with 25 per cent. HCl.

A method of detecting tin in distilled water was necessary because the distilled water used was carried in tin piping. The flame color test suggested by MEISSNER (6) and FEIGL (5) was used. To increase the sensitivity of the test, the test-water was reduced to one-half its volume by boiling. This concentrated sample was then acidified strongly with HCl, and a few pieces of zinc added. A test-tube containing cold water was used to stir this mixture; it was then placed in a nonluminous bunsen burner flame. In this test the blue mantle which forms around the test-tube in the presence of tin was absent, thus eliminating tin as a contaminant of the distilled water to one part in three million. Distilled water leached through the sand and recovered also failed to show the tin test.

To detect the presence of bivalent tin in plant tissues, the spot test method suggested by FEIGL (5) was modified. Thin sections of plant stems were acidified in 20 per cent. HCl by dropping the acid upon a glass slide holding the sections. A drop of dilute ferric chloride, and after a few minutes, a crystal of tartaric acid were added. When the tartaric acid crystal had dissolved, a drop of dimethylglyoxime was used on the sections, followed by ammonia. A red coloration, developed in the presence of reduced iron, indicates that stannous tin is present in the plant tissues.

FIG. 1. The effect of increasing concentrations of stannous sulphate on peas.

1. Control
2. 0.2 parts per million
3. 1.0 p.p.m.
4. 5 p.p.m.
5. 20 p.p.m.
6. 100 p.p.m.

Results

Figure 1 shows the effect on peas of stannous sulphate in concentrations from 0.2 to 100 p.p.m. The control, which was without tin sulphate, was slightly taller than plants grown in 0.2 p.p.m., although the difference is not marked since the branches and leaves were similar. One interesting result was that plants supplied with 0.2 p.p.m. of stannous sulphate showed increased root growth. Concentrations from 1 to 100 p.p.m. caused toxicity symptoms. These were reduced growth of internodes, reduction in size of roots, reduction in number of roots, absence of flowers in concentrations above 20 p.p.m., and yellowing of the lower leaves.

Figure 2 shows the effect of stannous sulphate on corn. The control, 0.2 p.p.m., and 1 p.p.m. plants were similar in structure, although the one receiving 1 p.p.m. appeared to be more vigorous. Other effects noted were a deeper chlorophyll green, and increased height. The plants which had received 0.2 and 1 p.p.m. of stannous sulphate (2 and 3, fig. 2) showed an increase in root growth. Greater concentrations (5, 20, and 100 p.p.m.) caused stunting and chlorosis, especially a yellowing of the lower leaves.

FIG. 2. The effect of stanous sulphate on corn.

1. Control
2. 0.2 parts per million
3. 1.0 p.p.m.
4. 5 p.p.m.
5. 20 p.p.m.
6. 100 p.p.m.

Figure 3 illustrates the effect of stannic chloride on the sunflower. The results with low concentrations (0.01, 0.05, and 0.1 p.p.m.) show that stannic chloride has a pronounced effect upon the growth of the sunflower. It is particularly effective on the roots, which were about twice the size of those

of the control. With additions of tin chloride above 1.0 p.p.m., stunting and reduced growth of leaves, internodes, and roots were observed. In concentrations of 100 p.p.m. sunflower was unable to reach a height of more than 12 in.

FIG. 3. The effect of stannic chloride in concentrations from 0.01 to 100 p.p.m. on the sunflower.

1. Control
2. 0.01 parts per million
3. 0.05 p.p.m.
4. 0.1 p.p.m.
5. 0.5 p.p.m.
6. 1 p.p.m.
7. 10 p.p.m.
8. 100 p.p.m.

Discussion

From the results obtained with trace concentrations of tin sulphate, it appears that its effects are limited to slight increases in the root growth of corn and pea. It also appears that corn and pea react specifically to this compound. In the corn plant 1 p.p.m. of stannous sulphate caused no unfavorable nor abnormal growth responses, while in the pea plant there was a decrease in the size, number of roots, and a stunting or top growth with this concentration. In concentrations of 100 p.p.m. the pea plant was barely able to exist; while corn, although stunted, was about the same size as the corn plants grown in much lower concentrations. As there are two nutrient ions to be considered, the slight stimulative effect on root growth might be caused by an increase in the concentration of the sulphate ion, but the added concentration in some cases is very small in proportion to the sulphate ion already present in the solution. The responses to high concentrations of stannous sulphate indicate the possibility that both sulphate and tin ions may cause changes in growth. It should be noted that in the higher con-

centrations there was a tendency toward precipitation of the tin salt. In the sunflower, trace additions of tin chloride caused striking changes in root growth, to about twice the size of the controls. The lack of marked favorable responses in root growth with additions of stannous sulphate to pea and corn may indicate that in some way the sulphate ion modifies the effectiveness of the tin ion, or that the plants are less sensitive to tin than sunflowers, or it may be that the chlorine ion in the presence of tin stimulates root development of the sunflower. Since it has been discovered very recently that tin increases the growth rate of certain aquatic animals, it seems more probable that the tin ion in very low concentrations is stimulatory to root growth. TOTTINGHAM (13) has reported some investigations with sodium and chlorine in which he found increased root development in the sunflower plant. He suggests that chlorine may function in some plants as a nutrient. This possibility must be kept in mind in interpreting the response of sunflowers to stannic chloride.

Conclusions

1. A method for the detection of bivalent tin in plant tissues has been devised.
2. In the sunflower, 0.01 and 0.05 p.p.m. of tin as tin chloride stimulates root growth.
3. Concentrations of tin, either as chloride or sulphate, in concentrations of 5 or more p.p.m. have toxic effects upon corn, pea, and sunflower.

The writer expresses his appreciation to Dr. C. A. SHULL for suggestions made in connection with this study.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. ALLISON, R. V., BRYAN, O. C., and HUNTER, J. V. The stimulation of plant response on the raw peat soils of the Florida everglades through the use of copper sulphate and other chemicals. Florida Agr. Exp. Sta. Bull. 190. pp. 33–80. 1927.
2. ARNON, D. I. Micro-elements in culture-solution experiments with higher plants. Amer. Jour. Bot. **25**: 322–325. 1938.
3. ———, and STOUT, P. R. Experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.
4. BRENCHLEY, W. E. The essential nature of certain minor elements for plant nutrition. Bot. Rev. **2**: 173–196. 1936.
5. FEIGL, F. Spot tests and color reactions as methods of microchemical work. Translated by JANET W. MATTHEWS. Nordemann Pub. Co., New York.

6. HAAS, A. R. C., and REED, H. S. Significance of traces of elements not ordinarily added to culture solutions for growth of young orange trees. Bot. Gaz. **33**: 77–84. 1927.
7. JONES, H. W., and SHIVE, J. W. The influence of iron in the forms of ferric phosphate and ferrous sulphate upon the growth of wheat in nutrient solution. Soil Sci. **11**: 93–101. 1921.
8. MEISSNER, H. Ein neuer Nachweis für Zinn. Zeitschr. Ann. Chem. **80**: 247–252. 1930.
9. MICHEELS, H. Sur les stimulants de la nutrition chez les plants. Rev. Sci. **14**: 427–429. 1906.
10. ————, and DE HEEN, P. Action de la solution colloidale d'étain sur des graines in germination. Bull. Acad. rayate Belg. Class des sci., no. 7. 1905.
11. SCHROPP, W., and SCHARRER, K. Wasserkultur Versuche mit der "A–Z" Lösung nach Hoagland. Jahrb. wiss. Bot. **78**: 544–563. 1933.
12. STEINBERG, R. A. Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*. Jour. Agr. Res. **51**: 413–424. 1935.
13. TOTTINGHAM, W. E. The rôle of chlorine in plant nutrition. Science n. s. **42**: 68. 1915.
14. YOUNG, R. S. Certain rarer elements in soils and fertilizers and their rôle in plant growth. New York (Cornell) Agr. Exp. Sta. Mem. 174. 1935.

THE LITHIUM METHOD OF MEASURING THE EXTENT OF CORN ROOT SYSTEMS

J. D. SAYRE AND V. H. MORRIS[1]

Introduction

Investigations of the root systems of corn plants which involve manually tracing the extent of individual roots are tedious and expensive. During the past 5 years a method of measuring the extent and distribution of corn roots through their absorption of lithium chloride has been under investigation. The method involves the placing of small quantities of the lithium salt in the soil at marked locations between the rows of corn and then spectrographically testing the tissue of nearby plants for the presence of lithium.

Lithium is not a normal constituent of soils except in rare instances. It is not toxic to the plants and is readily absorbed and accumulated in the plant in quantities which can be detected by a spectroscope. Very dilute concentrations may be identified spectroscopically, and no other element gives a line at 6708 Å. Its similarity in chemical properties to sodium and potassium probably accounts for its ease of absorption and accumulation in the plant, since these two elements are almost universally present in plant tissues.

The extreme simplicity of the method makes it useful in certain kinds of root investigations. It is recognized, however, that the method may be somewhat restricted in application.

Experimental procedure

The procedure of laying out the plots and conducting the experiments has varied from year to year. For most of the experiments the corn plants were spaced 14 inches apart in rows 42 inches apart, but in some tests the plants were planted in hills, in circles, or in spirals. In the spirals, the plants were spaced 14 inches apart on a line formed by unwinding a cord from a stationary drum 22 inches in diameter. These different arrangements were used to obtain uniform spacing of the plants at definite distances from the lithium spot. The lithium was placed midway between the rows or at the center of the spirals.

The lithium applications in most of the tests were made about the middle of July after the cultivations that might disturb them had been completed, and at the rate of approximately 2 grams of lithium chloride to 1,000 grams

[1] Department of Agronomy, Ohio Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating.

of soil. This was about the exchange capacity of the soil. A core of soil first was removed from each point of lithium application to a depth of 18 inches with a 4-inch post auger. The lithium chloride was dissolved in a small amount of water, sprinkled on the removed soil and thoroughly mixed with it. The treated soil then was returned to the hole and carefully packed to about the same consistency as before.

Each lithium spot was marked carefully. About September first all nearby plants were collected, their distance from the lithium spot was measured, and they were tested for the presence of the element. The test consisted of burning a small piece of tissue removed from one of the lower nodal plates in a Bunsen flame in front of a spectroscope. The presence of lithium was detected by a spectral line at 6708 Å. If the plant tissue contained lithium, the roots of that plant must have penetrated the core of lithium-treated soil and absorbed some of the salt. A negative test indiated that the roots had not extended to the lithium spot, or, if so, had failed to absorb the salt and transport it to the culm. As lithium is so easily absorbed by the plant and its detection in such small quantities is possible in the spectroscope, plants giving negative tests were considered as having no roots entering the lithium core. The results were recorded only as positive or negative, indicating the presence or absence of the element. No attempts were made to estimate the density of the roots in the lithium spot by measuring the quantity of lithium absorbed by the plant.

At the end of the season a 6-inch core of soil was removed from each lithium spot to ensure as complete removal of the material as possible. A series of spectroscopic tests showed that the lithium had not moved from the spot where it was placed. It enters into the base exchange complex of the soil and does not move with the soil water.

Experimental results

In 1932, preliminary tests showed that the method would work and lateral root extensions of more than 30 inches were found. The 1933 tests indicated about 2 grams of lithium chloride to 1,000 grams of soil as a proper amount to use. The 1934 experiments indicated a need for modifying the spacing of the plants in the row. With the plants spaced 14 inches apart in the row, few occurred at distances between 28 to 35 inches from the lithium source. As this distance is about the maximum root extension of many of the plants under the conditions obtaining at Wooster, more plants were needed in this interval for a good estimate of maximum extension. In 1935 and 1936 the plants were arranged in spirals around the lithium spots in order to locate them at more uniform intervals of space from the spot. Many other tests were conducted during these years, but not all of them can be discussed here.

TABLE I

SUMMARY OF ALL DATA INVOLVING SINGLE CROSSES AND A LITHIUM CONCENTRATION OF 2 GRAMS OF THE CHLORIDE TO 1,000 GRAMS OF SOIL

Year	Lithium test	Numbers of plants yielding positive or negative lithium tests at the distances from plant to lithium indicated								
		30–39 cm. or 11.8–15.4 in.	40–49 cm. or 15.7–19.3 in.	50–59 cm. or 19.7–23 2 in.	60–69 cm. or 23.6–27.2 in.	70–79 cm. or 27.6–31.1 in.	80–89 cm. or 31.5–35.0 in.	90–99 cm. or 35.4–39.0 in.	100–109 cm. or 39.4–42.9 in.	110–119 cm. or 43.3–46.9 in.
1933	Positive		6	12	2	5	0	0	0	0
	Negative		1	8	11	13	2	7	19	3
1934	Positive		10	15	9	2	0	0	0	
	Negative		0	0	3	8	0	10	9	
1935	Positive	12	31	35	21	12	0	1		
	Negative	0	2	20	24	32	38	43		
1936	Positive	37	15	17	14	21	18	5	2	0
	Negative	0	0	0	3	8	9	28	10	3
Total	Positive	50	62	80	46	40	18	6	2	0
	Negative	0	3	28	41	61	47	88	38	6
Percentage	Positive	100	95	74	48	40	28	6	5	0
	Negative	0	5	26	52	60	72	94	95	100

All of the data obtained in experiments involving single crosses and a lithium concentration of 2 grams of lithium chloride to 1,000 grams of soil have been summarized in table I. This table shows the distance from the plants to the lithium and the number of plants at each distance yielding positive or negative tests. The totals for the 4 years also are expressed on a percentage basis. All plants 40 cm., or 15.7 inches, from the lithium spot contained lithium, indicating their roots had penetrated the lithium core. No plants located as much as 110 cm., or 43.3 inches, from the lithium core contained lithium, and on the basis of this test the lateral extension of their roots is assumed to be less than this distance. Half the plants had roots 60 to 69 cm., or about 27 inches, in length.

The distances indicated by the method are not as great as would be expected from some results reported by others who actually dug the plants. Perhaps these results represent the extent of the mineral absorptive capacity of the corn plant rather than the maximum extension of roots. Fertilizer placement studies with corn indicate that the greatest mineral absorption occurs very near the plant. Cultural practices in much of the Corn Belt have shown that three plants to the hill with the hills space about 3½ feet each way, or equivalent stands drilled in rows 3½ feet apart, produce the largest yields of corn. These results seem to indicate that corn roots extend somewhat more than half the distance between rows. It is possible that root competition limits their extent laterally.

No tests were conducted on the maximum depth to which the root systems extended because of the shallow nature of the soils at Wooster, which have a tight clay subsoil at about 18 inches and a layer of shale at 2½ to 3 feet.

Ohio Agricultural Experiment Station
Wooster, Ohio

MONTHLY ABSORPTION OF NUTRIENTS FROM THE SOIL BY THE PERFECTION PIMIENTO PLANT[1]

H. L. COCHRAN AND L. C. OLSON

(WITH ONE FIGURE)

Studies on the absorption of nutrients from the soil by the Perfection pimiento plant were begun at the Georgia Agricultural Experiment Station in the Spring of 1939. Information of this nature is desirable because a knowledge of the kind and amount of nutrient absorbed at intervals throughout the growing season gives a better understanding of the fertilizer required for a more successful cultural program.

The soil on which the pimientos used in this work were grown is Cecil sandy clay loam. This is one of the most important soil types in the Piedmont section of Georgia, and pimiento culture is largely confined to this or a closely related soil type. The mean monthly rainfall for the 1939 growing season at Experiment, Georgia, where the experimental plats were located was 4.02 inches, and the mean temperature for the same period was 73.4° F.

The pimientos used in this study were fertilized at the rate of 600 pounds per acre with a mixed fertilizer analyzing 8 per cent. nitrogen, 8 per cent. phosphoric acid, and 6 per cent. potash. This fertilizer was applied in the row and mixed well with the soil several days before the plants were set out. At 30-day intervals, after transplanting, plants were cut off just above the soil line, dried at 75° C., and ground for chemical analysis. After the first month, the buds and fruits were separated from the plants for the analysis. In collecting the samples, care was taken to select uniform plants.

The results of the absorption of nutrients, calculated on an acre basis of 4,149 plants, are shown graphically in figure 1. At the end of the first month in the field, the entire acre of pimientos absorbed from the soil less than one-half pound of nitrogen and phosphoric acid, and even smaller amounts of potash, calcium, and magnesium. The absorption of nutrients during the second month was small, but there was an increase over the absorption for the first month. The largest quantity of all nutrients was absorbed during the third month. These findings are well in agreement with those of HESTER (2) for the tomato plant and those of CAROLUS (1) for the Irish potato. After the third month, there was a gradual decline in absorption. Larger quantities of nitrogen were absorbed than of any other nutrient. Calcium, potassium, magnesium, and phosphorus followed in this respective order as to quantity absorbed. Although phosphorus was absorbed the least of any,

[1] Published with the approval of the Director of the Georgia Agricultural Experiment Station as Journal Series Paper no. 73.

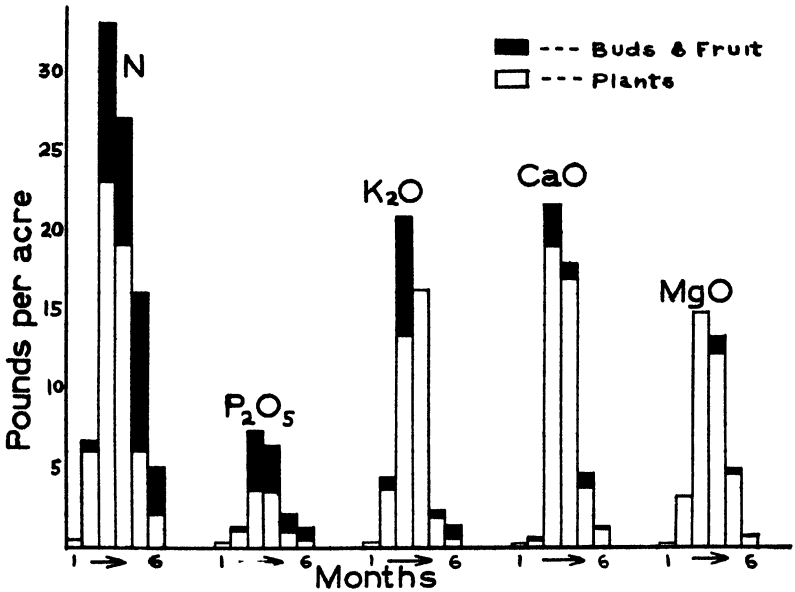


FIG. 1. The absorption nutrients by the Perfection pimiento plant at 30-day intervals during the growing season.

it is possible that rather large quantities should be included in the fertilizer mixture because of the rather high phosphate-fixing capacity of some of the pimiento soils, which makes some of the phosphate unavailable to the growing plant.

From the work completed thus far, it appears that ample plant nutrients should be made available to the pimiento plant, especially during the latter part of the second and throughout the third month after being set in the field. This may best be accomplished by placing most of the fertilizer under the plants a few days before setting and the balance as a sidedressing during late June. The second application should, perhaps, be supplemented with some extra nitrogen and potash.

DEPARTMENTS OF HORTICULTURE AND AGRONOMY
GEORGIA AGRICULTURAL EXPERIMENT STATION
EXPERIMENT, GEORGIA

LITERATURE CITED

1. CAROLUS, R. L. Chemical estimations of the weekly nutrient level of a potato crop. Amer. Potato Jour. **14**: 141–153. 1937.
2. HESTER, JACKSON B. The absorption of nutrients by the tomato plant at different stages of growth. Proc. Amer. Soc. Hort. Sci. **36**: 720–722. 1938.

NOTES

Seventeenth Annual Meeting.—Plans for the seventeenth annual meeting of the American Society of Plant Physiologists at Philadelphia in December, 1940, are well advanced. The meetings begin so late in the month that the first three days of the week, Monday to Wednesday, December 30, 1940, to January 1, 1941, have been chosen for the sessions. On Monday morning there is a symposium on Protoplasm; during the afternoon, there will be a joint session with Section G, A.A.A.S., and a parallel session for the reading of papers. This arrangement is made necessary by the large number of papers to be accommodated with time. On Tuesday morning, December 31, there is a joint session with the Phytopathological Society of America, and a parallel session for the presentation of contributions. In the afternoon, a symposium on Plant Nutrition is scheduled; this will be a joint meeting with the American Society for Horticultural Science. On Wednesday, January 1, 1941, there will be sessions, morning and afternoon, for reading of contributions. These will be divided into two groups for simultaneous meetings if necessary.

The headquarters will be at the Hotel Benjamin Franklin. The annual dinner, at which the award of the CHARLES REID BARNES life membership and the STEPHEN HALES award will be announced, has been set for Monday evening. Members and friends are urged to arrive early, and secure dinner tickets at once, in order that places may be reserved for the large number of attendants who will want to be present at these notable ceremonies. Dr. JOHN W. SHIVE will deliver the sixth STEPHEN HALES address at this time. The details as to the place of the annual dinner are uncertain, but it is hoped that the Athletic Club may be able to accommodate us with sufficient room. The program committee is to be congratulated on having its plans so well matured at this early date. There is every reason to expect a very large gathering, and members are advised to make early reservations; otherwise it may be difficult to secure hotel accommodations conveniently close to the meeting places.

Western Section.—The Western Section of the A.S.P.P. held its annual meeting at Seattle, with the summer meeting of the A.A.A.S., June 18–22, 1940. One of the outstanding features of this meeting was the symposium on Photosynthesis, which was conducted by Dr. H. A. SPOEHR, of the Carnegie Institution of Washington, D. C. Summarized briefly, this symposium included the following reports: HANS GAFFRON pointed out that, under certain conditions, oxygen is not liberated, but is used for the oxidation of organic hydrogen donors during photosynthesis. ROBERT EMERSON showed that the quantum number of the photosynthetic process is not 4, but approxi-

mately 10. WINSTON M. MANNING reported evidence that in diatoms carotenoids, in addition to chlorophylls, may be active in absorbing light energy for the photosynthetic processes. S. RUBEN reported experiments which indicated that plants reduce CO_2 in the dark by combining it with a large molecule of an as yet unidentified structure. E. D. MCALISTER showed the intimate relations between fluorescence and photosynthesis. WILLIAM ARNOLD showed that many chlorophyll molecules must be present in order to *insure* the photosynthetic process, although one individual molecule might do the work. Finally, C. B. VAN NIEL summarized the modern work on photosynthesis by presenting a scheme of the process. In this scheme CO_2 is reduced in the dark, and the H_2O (H_2A) is oxidized in the light.

In addition to this extremely valuable symposium, there was one on Aquatic Botany, at which Dr. GEORGE B. RIGG presided. GILBERT M. SMITH discussed the temperature of water in relation to the presence or absence of certain algae. JOHN RAPER presented evidence of the presence of sexual hormones in both algae and water molds. G. H. HOLLENBERG reported on the relative abundance of marine algae in relation to the presence of diatoms. LYMAN D. PHIFER discussed the distribution of marine diatoms; and J. VAN OVERBEEK showed that auxins are present in all marine algae investigated, and that traumatic acid and thiamin are growth factors for unicellular green algae. DAN BONNELL discussed the inter-relations of plant and animal life in certain fresh water lakes.

A third symposium dealt with the problems of Phosphate Nutrition. Dr. H. D. CHAPMAN was the leader of this symposium. PERRY R. STOUT illustrated by means of models how the phosphate is fixed on kaolinite and bentonite. ORLIN BIDDULPH showed how radio-active phosphorus "circulates" in the plant. The soil-plant relationships were discussed by W. T. MCGEORGE.

There were two half-day programs for short papers. Noteworthy among these were the following: J. B. BIALE reported that fungi produce emanations which affect the respiration of lemons; W. O. WILLIAMS discussed sugar in tracheids; L. A. HOHL showed that formic acid is not formed by yeast fermentation; ROBERT H. WILLIAMS gave an account of the reserve carbohydrates of algae; and ROBERT H. TSCHUDY reported studies on the control of growths on ship bottoms.

A trip to the oceanographic laboratories at Friday Harbor at the close of the meeting was very instructive, and from the number in attendance, it must be rated a great success. The total attendance at the meetings as a whole was estimated at about 100. At the annual dinner of the Western Section on Thursday evening, June 21, officers were announced for 1941 as follows: Chairman, A. S. CRAFTS, the University of California; vice-chairman, FRANK M. EATON, Rubidoux Laboratory, Riverside, California; secretary, J. VAN OVERBEEK.

Eighth American Scientific Congress.—The Eighth American Scientific Congress was held at Washington, D. C., in May, 1940. Its purposes were mainly to afford opportunities of friendly social contact between North and South American scientists, and to extend and consolidate the sense of unity and friendship existing between all of the American republics. Science, particularly, is friendly beyond the boundaries of nations. A number of papers on physiology and plant physiology were presented at the Congress, including the following: *Permeability in the nuclear membrane evidenced by intranuclear colloidal changes,* by WILLIAM R. DURYEE; *The nature of living cells as revealed by micro-operations,* by ROBERT CHAMBERS; *What the study of green plants has taught us concerning a probable mechanism of photosynthesis,* by O. L. INMAN; *Plant tissue cultures. A new technique in experimental science,* by PHILIP R. WHITE; and *Vitamins as growth substances for plants,* by WILLIAM J. ROBBINS. The contributions by our South American friends fell mainly in the fields of general botany, agriculture, and conservation.

The Congress was ably handled, and served its purposes admirably. The American Society of Plant Physiologists was represented at the Congress by Dr. CHARLES O. APPLEMAN, Professor of Plant Physiology and Dean of the Graduate School, at the University of Maryland.

Life Membership Committee.—The seventeenth award of the CHARLES REID BARNES life membership will be made at Philadelphia, the announcement being a part of the festivities of the annual dinner. President FRANK P. CULLINAN has named the committee which is to have the privilege of selecting the recipient of the award. The membership of the committee is as follows: Chairman, Dr. H. R. KRAYBILL, Purdue University; Dr. C. J. LYON, Dartmouth College; Dr. PAUL J. KRAMER, Duke University; Dr. F. H. STEINMETZ, The University of Maine; and Dr. W. E. TOTTINGHAM, The University of Wisconsin.

Finances.—At the beginning of the year 1940 it seemed that the American Society of Plant Physiologists might be facing a very trying year because of the widespread economic disturbance throughout the world. Thanks to the efforts of our secretary, Dr. W. E. LOOMIS, and great numbers of American plant physiologists who understood the situation and acted upon their knowledge, we have come through the year in sound, solvent condition. Every society that undertakes to publish a scientific journal is at the mercy of its income. Few of our societies devoted to science have adequate financial reserves and endowments to assist them in times of crisis. The American Society of Plant Physiologists has tried consistently to build for the future, as well as to take care of the present. Many individuals have contributed thought, service, and money in order that we might grow toward

independence. The purchase of life memberships, and patronage have aided much; in addition the Society has set up machinery which has a tendency to strengthen our hands, and to provide a part of the overhead which is unavoidable in our existence.

Earlier in the year a report was promised on the endowments of the Society, as of the close of the 1939–40 fiscal year. These funds are listed as to principal, and available funds, at face value as follows:

BARNES Life Membership Endowment Fund,	Principal,	$3000.00
	Available,	52.30
STEPHEN HALES Endowment Fund,	Principal,	2000.00
	Available,	144.93
Life Membership, and General Endowment,	Principal,	3723.81
Total Endowments,		$8921.04

In addition to these funds there is a reserve, containing securities of unknown value. These have a face value of $1000.00, but represent reorganization properties, and cannot be given any definite value at present. If these were sound bonds, the endowments would be approaching a value of $10,000.00, obtained over a period of 15 years. May we not hope that an increasing number of our members may be challenged by these figures into joining with those who have given generously in the past? Gifts of any size are welcome; and one may become a life member or a patron at any time. There are seven patrons; there should be several times this number. And no better investment can be made by a young man than a life membership. In 20 years one pays the same amount in dues, and there is no gain to the Society's permanent funds. In 40 years, from age 25 to 65, the dues are double a life membership; and in addition to 100 per cent. interest to the individual, the Society finally has an increase in its permanent funds. There is also some satisfaction in feeling that one has contributed toward the permanent welfare of science. The treasurer will appreciate any contribution that may be made toward the building of permanency for the American Society of Plant Physiologists.

Errata.—A few errors in volume 14 of PLANT PHYSIOLOGY have been reported by authors. We appreciate the cooperation of those whose papers appear in the journal, and urge all to proofread and send in reports of errors observed. Please note the following:

Page 311, formula, for "Vg," read V_g, and for "a" read α.
Page 643, line 5 from bottom, for "weks" read weeks.
Page 645, citation 1, for "1037" read 1937.
Page 688, line 16, for "figure 3, curve d" read figure 2, curve D.

Hormone Information.—A mimeographed report, comparing the suitability of United States grown oats for the Avena test, has been prepared

by J. VAN OVERBEEK, California Institute of Technology, Pasadena, California. All Experiment Stations engaged in oat breeding were requested to send samples of oats for comparative tests. Victory oat, and Markton, proved to be most valuable for the auxin determination. Nearly 40 tests are reported, arranged in descending order of sensitivity. This valuable report may be obtained from the California Institute of Technology on request. It will aid much in the standardization of technique of making quantitative auxin measurements. Address requests to Dr. VAN OVERBEEK.

Naturalist's Directory.—The 32nd biennial edition of The Naturalist's Directory will be published in September, 1940. This directory contains the names, addresses, and special subjects of interest of naturalists in all parts of North and South America, as well as a list of periodicals and Natural History museums. The price of the directory is $2.50, and it is published by The Naturalist's Directory, Salem, Massachusetts.

Plantation Crops.—Technical Communication no. 13, of the Imperial Bureau of Horticulture and Plantation Crops, East Malling Research Station, near Maidstone, Kent, England, bears the title *Vegetative Propagation of Tropical and Subtropical Plantation Crops.* It has been compiled by G. ST. CLAIR FEILDEN and R. J. GARNER. It deals with the vegetative propagation of some 55 plantation crops. Major crops, such as rubber, coffee, cacao, etc., have received the attention of technical experts, and the foreign literature has been thoroughly combed for details of propagation of less familiar crops. One most valuable feature is the section devoted to methods used in vegetative propagation. The descriptions are supported by simple, clear, line drawings of some 17 types of graft, and 7 types of budding commonly used in vegetative propagation. Tropical workers will be interested in the illustrated account of the construction of loosely woven potting baskets which have been found useful as a substitute for pots in tropical nursery work. References to the original literature immediately follows the discussion of the propagation of each particular crop.

This valuable bulletin is priced at 3 shillings 6 pence. Orders may be addressed to the Imperial Bureau, East Malling, Kent, England.

French-English Dictionary.—A year ago we had the privilege of announcing a dictionary of German scientific terms by LOUIS DEVRIES, of the Iowa State College. We now are happy to announce that a companion volume, *French-English Science Dictionary,* by the same author, has just been published by the McGraw-Hill Book Co. It is an excellent volume for students and professors who have to read English scientific literature, or who must prepare for the modern language requirements associated with higher degrees. It makes no pretenses to completeness, but the vocabulary

is well-chosen, and covers the ground in a most satisfactory way. If students in reading meet words which they cannot find in the dictionary, the author requests that such words be sent to him for possible inclusion in future editions. The author and publishers deserve great commendation for the service they have rendered. The book should receive a wide and enthusiastic reception. It is an excellent desk companion, and its possession will certainly encourage more general reading of French scientific literature. The list price is $3.50.

Botany.—The third edition of HOLMAN and ROBBINS, *Elements of Botany*, has been published by John Wiley and Sons, New York. The main changes concern the recent advances in plant physiology, growth substances, culture solutions, erosion physiology, photoperiodic control of blooming, artificial pollination, self sterility, efficiency of photosynthesis, disease and weed control, economic value of plant products, and induction of chromosome changes. The quoted price is $2.75 per copy.

GENERAL INDEX[1]

A

B

[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The H. W. Wilson Co., New York.

D

Q

R

T

U

V